The Synthesis of Biologically Interesting Pseudopeptides

by

Zoe Anderson

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry

University of Warwick, Department of Chemistry

August 2014

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SCHEMES

LIST OF TABLES

LIST OF EQUATIONS

ACKNOWLEDGEMENTS

The EPSRC is gratefully acknowledged for providing the funding for this project.

My sincere thanks go to my supervisor, Dr David Fox, for many reasons. Principally, I would like to thank him for the chance to undertake a PhD, and for allowing it to diverge so far from the original aims. I can honestly say I have thoroughly enjoyed the past four years, and Dr Fox's humour, patience and apparently encyclopaedic knowledge of interesting bits of chemistry have played a large part in this.

I would also like to thank the other members of the Fox group, both past and present, who have provided me with support, ideas, and extended conversations in the pub, many of them about chemistry. Thanks must also go to my friends and family outside chemistry who have put up with a lot of conversations about things they neither know, nor care, very much about, despite my best efforts. Special thanks go to Nick Dunn for keeping me supplied with tea, food and beer, especially through the final stages of writing up.

Many thanks must go to the technical staff in the department- to Dr Lijiang Song, Mr Philip Aston and Dr Rebecca Wills for the mass spectrometry service, and to Dr Ivan Prokes, Mr Edward Tunnah and Mr Robert Perry from the NMR department. The data they obtained for me was invaluable, and I am extremely grateful for all their help. I would also like to thank Dr Lijiang Song and Professor Gregory Challis for the opportunity to work on azolemycin, which proved to be a fantastic final year project.

DECLARATION

All of the work carried out in this thesis is original research carried out at The University of Warwick between October 2010 and July 2014. I declare that the material described that is not original has been identified and appropriately referenced. I certify that the material within this thesis has not been submitted for a degree at any other university.

ABSTRACT

This thesis begins with an introduction to inflammation and current anti-inflammatory drugs in general, and the new small molecule somatotaxins developed by Fox and colleagues in particular. Chapter 1 describes the evidence used to propose the existence of structurally similar *C*-terminal lactam peptides that could potentially provide a novel anti-inflammatory pathway *in vitro*.

Chapter 2 describes the synthesis of some *C*-terminal lactam peptides using solution phase peptide synthesis. Both linear and block syntheses were used. This chapter also describes some of the problems encountered using peptide coupling agents and our attempts to overcome them.

Chapter 3 describes our investigations into thioacids for the block synthesis of peptides. The use of isoleucine in model peptides for the quantification of epimerization at the *C*-terminal amino-acid is also illustrated, and a series of NMR experiments were performed to allow us to differentiate between the relative stereoisomers of isoleucine residues in compounds.

Chapter 4 describes the use of these ^{1}H NMR experiments to predict the relative stereochemistry of a *C*-terminal isoleucine residue in the natural product, azolemycin A. The first total synthesis of azolemycins A and B, and a number of analogues, is then described.

ABBREVIATIONS

7-TM	Seven transmembrane
Å	Angstrom
AIDs	Acquired immunodeficiency syndrome
Anal.	Analysis
atm	Atmospheres
aq.	Aqueous
Bn	Benzyl
Boc	Di-*tert*-butyl dicarbonate
BSCI	Broad spectrum chemokine inhibitor
c	Concentration
Calc.	Calculated
CBz	Carboxybenzyl
CLR	C-type lectin receptor
COSY	Correlation spectroscopy
COX	Cyclooxygenase
CTLP	*C*-Terminal Lactam Peptide
d	Doublet
DAST	(Diethylamino)sulfur trifluoride
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	*N,N'*-Dicyclohexylcarbodiimide
DEAD	Diethyl azodicarboxylate
Deoxy-Fluor	Bis(2-methoxyethyl)aminosulfur trifluoride
DEPT	Distortionless Enhancement by Polarisation
$(DHQD)_2PHAL$	Hydroquinidine 1,4-phthalazinediyl diether
DIBAL	Diisobutylaluminium hydride
DIPEA	Diisopropylethylamine

DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
EDCI	3-(Ethyliminomethyleneamino)-*N*,*N*-dimethylpropan-1-amine
EDTA	Ethylenediaminetetracetic acid
equiv.	Equivalents
ESI	Electrospray ionisation
FAD	Flavin adenine dinucleotide
FMoc	Fluorenylmethyloxycarbonyl chloride
FT	Fourier transform
GH	Growth hormone
GPCR	G-Protein coupled receptor
HATU	*O*-(7-Aza-benzotriazol-1-yl)-*N*, *N*, *N*, *N*'-tetramethyluronium hexafluorophosphate
HIV	Human immunodeficiency virus
HOBt	1-Hydroxybenzotriazole
HMBC	Heteronuclear multiple-bond correlation spectroscopy
HMPA	Hexamethylphosphoramide
HMQC	Heteronuclear multiple-quantum correlation spectroscopy
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
IL	Interleukin
lit.	Literature
Ltd	Limited
M	Molar
MCP-1	Monocyte chemotactic protein 1
m.p.	Melting point
Ms	Methanesulfonyl
NMM	*N*-Methylmorpholine

NSAID	Non-steroidal anti-inflammatory drug
oct.	Octet
P450	Cytochrome P450
PhD	Doctor of philosophy
ppm	Parts per million
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
PyBrop	Bromo-tris-pyrrolidinophosphonium hexafluorophosphate
Pyr.	Pyridine
q	Quartet
quat.	Quaternary
quin.	Quintet
s	Singlet
sat.	Saturated
sept.	Septet
sext.	Sextet
SST	Somatostatin
sstr	Somatostatin receptor
t	Triplet
T	Temperature
TBAF	*tert*-Butylammonium fluoride
TBDMS	*tert*-Butyldimethylsilyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilyl
TMU	Tetramethylurea
TNF	Tumour necrosis factor
TOF	Time of flight
Ts	4-Toluenesulfonyl

AMINO-ACIDS

A – Ala – Alanine

C – Cys – Cysteine

D – Asp – Aspartic acid

E – Glu – Glutamic acid

F – Phe – Phenylalanine

G – Gly – Glycine

H – His – Histidine

I – Ile – Isoleucine

K – Lys – Lysine

L – Leu – Leucine

M – Met – Methionine

N – Asp – Asparagine

O – Orn – Ornithine

P – Pro – Proline

Q – Gln – Glutamine

R – Arg – Arginine

S – Ser – Serine

T – Thr – Threonine

V – Val – Valine

W – Trp – Tryptophan

Y – Tyr – Tyrosine

CHAPTER 1: INFLAMMATION

Inflammation is a natural, non-specific, immune response to infection or tissue injury, leading to characteristic physical symptoms, namely localised heat, swelling, redness and pain.[1] Inflammation is generally considered to be beneficial, as it protects an organism against sustained damage and infection.

1.1 THE INFLAMMATORY PROCESS

The innate immune system has an important role in inducing the acute inflammatory response.[2] When physical damage to tissue occurs, dead and dying cells release or expose cell-derived molecules known as damage-associated molecular patterns (DAMPs).[3] These are located in both intra- and extracellular spaces including plasma membranes, mitochondria and other intracellular molecules, and the extracellular matrix.[4] In contrast, pathogen-associated molecular patterns (PAMPs) and non – pathogenic microbe-associated molecular patterns (MAMPs) are not derived within the body, but are instead a diverse group of evolutionarily conserved molecules expressed by a range of pathogens, including nucleic acids, surface proteins and carbohydrates (Figure 1).[5, 6]

Both PAMPs and DAMPs are recognised by pattern-recognition receptors (PRRs) expressed by a number of cells including macrophages, dendritic cells, and cells not directly involved with inflammation such as epithelial and endothelial cells and fibroblasts.[7] These PRRs include toll-like receptors (TLRs), NOD-like receptors (NLRs), C-type lectin receptors (CLRs) and RIG-I-like receptors (RLRs) and are located in both the transmembrane and cytoplasmic regions of the cells. Upon binding PAMPs and DAMPs, PRRs signal the presence of damage or infection and induce a pro-inflammatory response and, if pathogens are present, an antimicrobial response.[8] This generally results in the upregulation of the transcription of genes encoding pro-inflammatory cytokines, chemokines, type 1 interferons (IFNs) and many other proteins.[7]

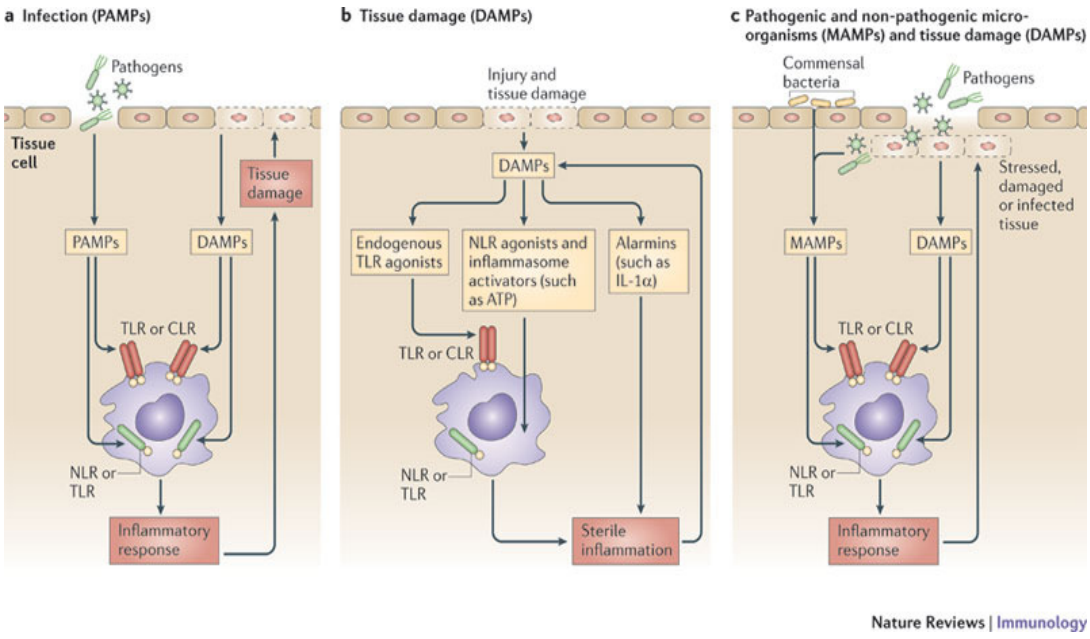


Figure 1: Mechanisms for the induction of inflammation (reproduced from Mills, K. H. G., Nature Reviews Immunology, 2011)[6]

The induction of inflammation elicits the production of a diverse range of inflammatory mediators. These lead to the three broad, overlapping phases of inflammation: a vascular response, a cellular response and a chemical response.

The vascular response is initiated by a variety of inflammatory mediators. These are released by the degranulation of mast cells and include vasoactive amines, such as histamine and serotonin, and vasoactive peptides including kinin, thrombin and plasmin.[9] These molecules induce localised vasodilation and an increase in vascular permeability. This is designed to allow rapid influx of anti-inflammatory cells to the area, as well as the dilution of any toxic pathogenic products and the drainage of fluid out of the vessels. It is also this that causes the physical symptoms of swelling and redness, as blood rushes to the area and fluid collects, causing oedema.

The cellular response is predominately involved in the recruitment and activation of leukocytes to the site of trauma. Leukocytes, often called white blood cells, are normally present in the blood and, following an inflammatory trigger, they must be diverted to the source of infection or damage, which is usually outside of a blood

vessel. There are a number of chemical mediators that are involved in the initial recruitment and directing of the leukocytes (Figure 2). Activation of the endothelial cells by cytokines such as IL-1β and TNF-α causes the expression of adhesion molecules, leading to leukocytes binding loosely to the blood vessel walls.[10] The selectin family of molecules is then responsible for *rolling* the leukocytes along the walls. Once rolling, chemokines activate integrin molecules on the leukocytes, and the cells are activated to bind more firmly to the endothelial cells.[11] Following binding, the leukocytes then migrate across the endothelium into the affected tissue. This is mediated by a number of molecules found on the endothelial cell borders, including platelet endothelial cell adhesion molecule-1 (PECAM-1, or CD31) and vascular cell adhesion molecule-1 (VCAM-1).[12] These help to bind the leukocytes as they squeeze between the endothelial cells into the extracellular space.

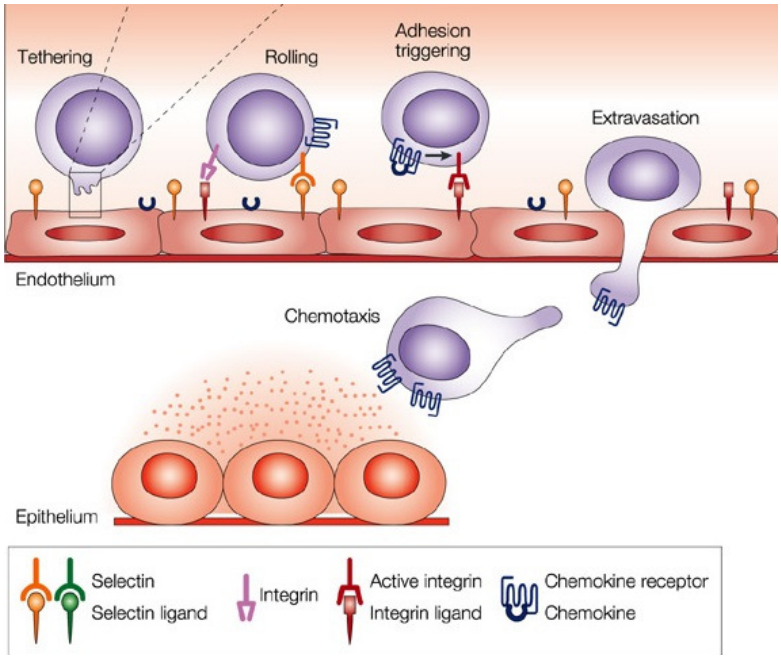


Figure 2: Recruitment and activation of leukocytes(reproduced from Kunkel E. J. *et al*, Nature Reviews Immunology, 2003)[13]

Once outside of the blood vessel, chemotactic molecules produced by neutrophils at the site of infection or injury form a chemical gradient that guides the leukocytes to the area. There is a diverse array of chemotactic mediators linked to inflammation including:

- C5 fragments, produced by cleavage of the C5-glycoprotein at the site of infection[14]
- N-formyl peptides, from the processing of bacterial proteins[15]
- Leukotriene B_4 from the metabolism of arachidonic acid
- Cytokines, including TNF-α, IL-8 and MCP-1.[16]

Once outside the blood vessel and at the site of infection, leukocyte differentiation is triggered. Neutrophils are the first leukocytes to gather, recognize and engulf pathogens at the site of infection. They are activated by a number of cytokines and chemokines, and this leads to molecular changes that extend the lives of the cell allowing them to effectively tackle infection. As well as releasing further pro-inflammatory chemokines, they also release a number of proteases and reactive oxygen species (ROS) in order to kill phagocytosed cells. [17] Once they have engulfed and destroyed pathogens, apoptosis is induced. Apoptotic cells release further signals that attract macrophages, which engulf them. This prevents the toxic cell contents being released during necrosis.[18, 19]

At the site of infection, monocytes differentiate into either mature macrophages or immature dendritic cells.[20] Macrophages are the main source of cytokines and growth factors that affect the local cell environment and induce tissue repair at the site of injury. [21] In addition, as previously mentioned, macrophages are involved in the phagocytosis of apoptotic neutrophils. It appears that the ingestion of dead neutrophils may trigger the egress of macrophages from the site of inflammation to the local lymph system, where they are removed.[19]

In addition to the vascular and cellular responses to inflammation, there is also a concurrent chemical response. A huge array of different chemical mediators is released during the acute phase of inflammation. In addition to those already

mentioned (vasoactive amines and amides, and kinins) there are a number of other notable groups of mediators.

1.2 THE COMPLEMENT SYSTEM

This is a collection of more than 30 soluble or membrane proteins that make up part of the innate immune system; that is, it does not adapt to new threats, but can be recruited by the adaptive immune system.[22] The complement system is widely distributed throughout the blood plasma, and is activated locally to help trigger anti-bacterial and anti-inflammatory responses.[23]

1.2.1 ARACHIDONIC ACID METABOLITES

Archidonic acid is a 20-carbon unsaturated fatty acid derived from the phospholipid bilayer. It is metabolised by a variety of enzymes, namely cyclooxygenases (COX enzymes) and lipoxygenases, to give a range of prostaglandin and leukotriene products that are intimately involved in the propagation of an inflammatory response (Figure 3).[24]

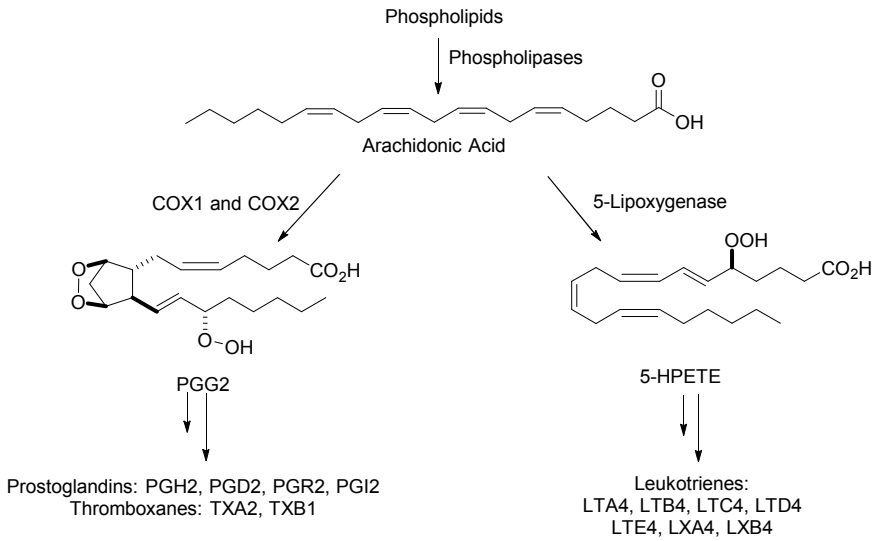


Figure 3: The metabolism of arachidonic acid to prostaglandins and other derivatives[24]

Prostaglandins are products of the COX enzymes, and their production is linked to sensitisation of pain receptors, vasodilation, increased vascular permeability, chemotaxis, and platelet aggregation.[25] Leukotrienes are produced by 5-lipoxygenase

and act on vasodilation and constriction, chemotaxis of neutrophils and vascular permeability.

1.2.2 CYTOKINES

Cytokines are small proteins (8 - 40kDa) that are expressed by nearly every cell.[26] Depending on their source, they can regulate both pro- and anti-inflammatory responses. Broadly, there are three main groups of cytokines: Interleukins, TNF and chemokines.

Interleukins and TNF are produced mainly by mast cells, endothelial cells and macrophages at the site of infection.[27] The major interleukins associated with inflammation are IL-1 and IL-6. IL-1 is involved in prostaglandin synthesis, growth and differentiation of T and B cells and growth of fibroblasts,[28] while IL-6 is linked to macrophage and B cell differentiation and antibody formation, and both IL-1 and IL-6 are also linked to the induction of acute phase proteins.[29] TNF primarily acts on endothelial cells, leading to leukocyte accumulation and adhesion, increased vascular permeability and endothelial cell hypertrophy.[30] Both IL-1 and TNF also stimulate chemotactic factors, as well as acting on target organs e.g. acting on the brain to cause fever, and the bone marrow to promote leukocyte production. [31]

1.2.3 CHEMOKINES

Chemokines are a range of small proteins involved in regulating the traffic of various cells to different tissues. They are intimately involved in the process of inflammation, as they are released at the site of injury or infection and direct leukocytes and other macrophages to the area, leading to the physical response that is known as inflammation.[32] They act by binding to specific 7-transmembrane G-protein coupled receptors (GPCRs) located on the surface of target cells.[33] These receptors all contain a conserved disulphide bridge between cysteine residues in the first two extracellular loops, which is essential to maintain the structure of the receptor for ligand binding.[32, 34]

There are at least 46 known chemokines, all small peptides, generally with four characteristic conserved cysteine residues.[35] The relative position of the first two cysteine residues is used to group the chemokines into families. There are two major chemokine groups, CC and CXC, and two minor groups, CX3C and C, in which C denotes a cysteine residue, and X a non-cysteine amino acid.[36] Of the two major groups, CC chemokines act to induce monocyte proliferation, while CXC chemokines are chemoattractants for neutrophils.[37, 38]

As chemokines are separated into families, so are the receptors according to their particular chemokine preference. There are at least 7 described as CXCR, 11 as CCR, and 1 each of either XCR or CX3CR respectively.[39] Within each family of receptors, each receptor can bind to several chemokines, and each chemokine can bind to several receptors, though this promiscuity cannot cross the family boundaries.[40]

1.3 RESOLUTION OF INFLAMMATION

If the inflammatory stimulus is successfully removed, the initial acute phase is actively terminated and the emphasis moves to removing dead cells and repairing damaged tissue, in a process known as the resolution phase. At this stage, normally a few hours after the start of an inflammatory event, a switch from prostaglandins and leukotrienes to lipoxins is instigated, neutrophil recruitment stops and apoptosis of leukocytes begins. If the inflammatory stimuli persist, or the normal mechanism for inflammation resolution is disrupted, chronic inflammation can occur. This results in the constant renewal and degradation of the tissue, and is associated with ongoing pain, swelling and loss of function. Infiltration by neutrophils and macrophages is primarily connected to acute inflammation, while chronic inflammation tends to involve the influx of plasma cells and T-cells.[1] Persistent, inappropriate inflammation has been linked to a variety of diseases, including HIV, atherosclerosis, asthma and rheumatoid arthritis.[41-45]

1.4 CURRENT ANTI-INFLAMMATORY DRUGS

Inflammation is a highly complex process, and as such is difficult to treat. Anti-inflammatory drugs tend to act by blocking early pro-inflammatory pathways, preventing the physiological effects of swelling and pain.

Historically, treatments for inflammation are broadly split between two groups of molecules-non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. NSAIDs, a class of drug that includes aspirin, ibuprofen and naproxen amongst others, are commonly used to treat pain and inflammation from arthritis, headaches, and a variety of musculoskeletal problems. They act on the cyclooxygenase (COX) enzymes in the body. Normally, these enzymes catalyse the oxidation of arachidonic acid to give prostaglandin-H_2, the precursor to the pro-inflammatory agents prostaglandins and thromboxane.[46] Aspirin irreversibly binds COX enzymes, while other NSAIDs such as ibuprofen, bind reversibly.[47]

There are two main classes of COX enzymes, COX-1, COX-2, and traditional NSAIDs such as aspirin are non-selective. COX-1 is widespread in the body, and amongst other things is responsible for the production of gastro-protectional prostaglandins. Inhibition of COX-1 by NSAIDs can therefore be detrimental, leading to gastric ulcers and renal toxicity.[48] COX-2, in contrast, is an inducible enzyme, and is produced in response to endotoxins, mitogens and cytokines in cells responsible for the production of inflammatory prostaglandins.[49] However, the development of a COX-2 specific anti-inflammatory has been hampered by the potential side-effects. Vioxx, a COX-2 inhibitor produced by Merck, was withdrawn from used in 2004 after studies linked it to an increased risk of cardiovascular events.[50]

Corticosteroids refers to both mineralocorticoids, which are steroid hormones linked to regulating salt levels,[51] and glucocorticoids. Glucocorticoids consist of both naturally occurring hormones and synthetic analogues, and are used to treat a range

of inflammatory disorders, most commonly as the first line of treatment for asthma.[52] They act by binding to the glucocorticoid receptor, which is present in most tissues.[53] When stimulated by the endogenous ligand cortisol (Figure 4), the receptor directly inhibits a variety of anti-inflammatory pathways through the production of anti-inflammatory molecules such as MAPK phosphatase I and by blocking the transcription of inflammatory molecules such as prostaglandins and calcium kinase II.[54]

A range of synthetic glucocorticoids have been developed that exhibit greater potency than the endogenous ligand, cortisone. An inhaled steroid preparation is manufactured by GlaxoSmithKline under the brand name Advair (Figure 4), and is a mixture of a glucocorticoid (fluticasone propionate) administered with a bronchodilator (salmeterol xinafoate).[55] It was the sixth best selling prescription drug in the United States of 2013, with sales of over $3.7 billion.[56] However, there have been concerns that long term use may exacerbate asthma-related deaths, though this is likely to be due to the salmeterol component rather than the steroid.[57]

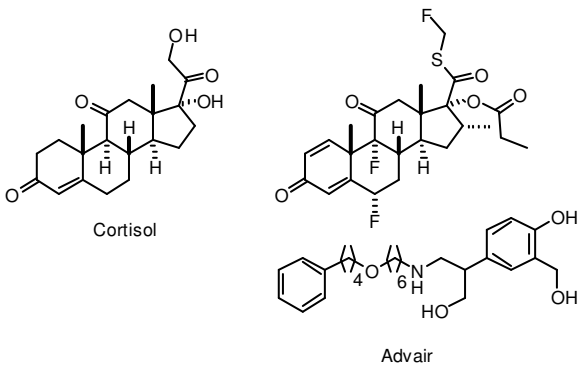


Figure 4: The structures of cortisol and Advair[58]

Potential side effects associated with glucocorticoid drugs include localised atrophy of the skin and muscles, delayed wound healing, increased risk of infection and a number of endocrine, gastrointestinal and cardiovascular disorders.[59] Limiting the dose and length of treatment are generally recommended for these reasons. In addition, a minority of inflammatory diseases commonly treated with steroids appear

to be resistant to their effects. Other diseases related to inflammation, including cystic fibrosis, chronic obstructive pulmonary disease and interstitial fibrosis, seem to be almost completely resistant to treatment by steroids. [60]

Drugs that inhibit cell migration molecules, including chemokines, are very attractive pharmaceutically. To begin with, cell migration molecules target G-protein coupled receptors (GPCRs). GPCRs tend to be readily inhibited by small organic molecules, which in turn tend to be orally available. In addition, chemoattractant molecules are abundant, suggesting they must be prominent in precisely directing leukocyte traffic, and indeed inhibition of chemoattractants has been shown to be highly effective against a range of anti-inflammatory agents in a range of models. [61-63]

1.5 SPECIFIC CHEMOKINE INHIBITORS

Inhibition of chemokines is considered to be a promising method to interfere with cellular recruitment, and hence induce anti-inflammatory effects.[64] Upregulation of chemokine receptors and their ligands has been associated with a number of inflammatory diseases, including rheumatoid arthritis,[65] angiogenesis,[66] transplant rejection,[67] and HIV/AIDS.[68]

Attempts to treat these diseases using specific chemokine receptor inhibitors have met with mixed success. Both CCR5 and CXCR4 receptors can act as co-receptors for entry of HIV-1 strains into cells, and therefore inhibition of either could provide promising new classes of antiviral treatments for HIV infection.[69] A number of CCR5 antagonists for the treatment of HIV/AIDS have been developed (Figure 5), including maraviroc (Pfizer),[70] vicriviroc (Schering-Plough and Merck),[71] and aplaviroc (GlaxoSmithKline).[72]

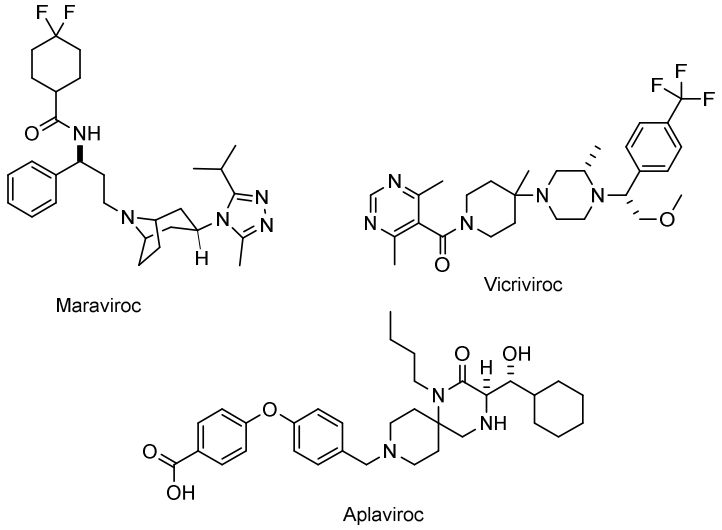


Figure 5: CCR5 antagonists

Studies of aplaviroc were discontinued due to concerns about heptotoxicity,[73] and Merck announced it would not seek federal approval for vicriviroc after it failed to show superior results compared to the optimised regimes in Phase III trials.[74] However, maraviroc was approved by the European Commission for the treatment of HIV in 2007,[75] and has performed well in Phase I and II trials.[76]

Several CXCR4 antagonists have been developed to date (Figure 6). Plerixafor reached Phase I trials and AMD11070 reached Phase II trials for the treatment of T-tropic HIV, but both were discontinued.[77] However, CXCR4 antagonists are also known to mobilize a variety of leukocytes, most notably hematopoietic stem cells.[78] This has led to the a number of CXCR4 antagonists being developed for other purposes including enhancement of current chemotherapy treatments, and, in the case of plerixafor, the treatment of non-Hodgkin's lymphoma (NHL) or multiple myeloma (MM).

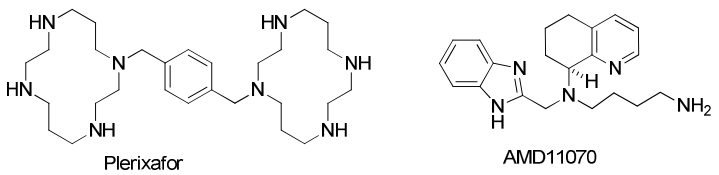


Figure 6: CXCR4 inhibitors[79]

CCR9 is primarily expressed on T cells migrating within the digestive system, and is present in high concentrations in the intestinal mucosa.[80] It has one known ligand, CCL25, which is also highly expressed in the intestine and thymus.[81] Overexpression of both CCR9 and CCL25 has been linked to the incidence of inflammatory bowel diseases, particularly Crohn's disease.[82, 83] GSK-1605786 (CCX-272; Traficet-EN; vercirnon; Figure 7) is a small molecule CCR9 antagonist being developed by GlaxoSmithKline, under license from ChemoCentryx, for the treatment of IBDs, including Crohn's disease and celiac disease.[84] It has shown promising results in the treatment of Crohn's disease, entering Phase III clinical trials in 2013.[85]

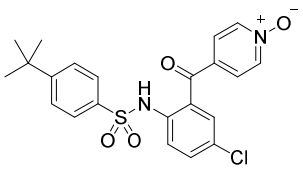


Figure 7: CCR9 antagonist

The complex nature of chemokine receptors and their multiple ligands, including the high level of redundancy, mean that targeting a specific receptor for a particular disease can be difficult. Animal models, including gene-deficient mice, are often used to mimic diseases, but often drugs that are effective in animal models show no activity in human trials. This has been particularly obvious in the antagonism of single chemokine receptors for the treatment of rheumatoid arthritis that showed promise in animal trials that did not translate to human treatment.[86-88]

The level of redundancy between chemokines and their receptors means that drugs must be targeted to cell migration molecules carefully. Too great a selectivity and it is likely the drug might be ineffective, as a number of chemokines may promote pro-inflammatory molecules in parallel by signalling through a number of different receptors. Equally, as the full complexity of chemokine receptors is not yet understood, too broad a selectivity and the drug risks unknown immunocompromising side effects.[63, 89]

1.6 BROAD SPECTRUM CHEMOKINE INHIBITORS

In 1991, Grainger *et al.* published the discovery of "Peptide 3", an amino-acid sequence isolated from monocyte chemoattractant protein-1 (MCP-1), which was shown to inhibit a wide range of chemokines *in vitro*.[90] MCP-1, also known as CCL2, is a member of the CC chemokine subgroup, and is a 13kDa protein expressed either constitutively or after induction by a number of pro-inflammatory triggers.[91] MCP-1 attracts and activates monocytes by binding to a number of GPCRs, including CCR2,[92] CCR11,[93] and the Duffy antigen receptor for chemokines (DARC). This last is unusual in that it binds a range of chemokines from both the CC and CXC subgroups.[94] Peptide 3 corresponded to the amino-acids 51-62 of MCP-1 (EICADPKQKWVQ, single letter amino-acid code), and inhibited leucocyte migration induced by MCP-1, MIP-1α, RANTES, IL-8 and stromal cell-derived factor-1 α (SDF- α) at the 2-10 μM range. However, it did not inhibit the migration of leukocytes induced by non-chemokine chemoattractants, including tumour growth factor beta-1 (TGF-β1) and *N*-formylmethionine leucyl-phenylalanine (fMLP). As such Peptide 3 was the first synthesised pan-chemokine inhibitor, but was unsuitable for *in vitro* studies due to poor solubility, short plasma halflife and susceptibility to enzymatic degradation.

In an attempt to combat this, a cyclic peptide, NR58-3.14.3, was synthesised using the unnatural D-amino acid equivalents (Figure 8). This afforded a BSCI with a backbone unaffected by proteolytic digestion, and vastly improved potency of around 1000 times that of Peptide 3.[15] NR58-3.14.3 was shown to be a promising anti-inflammatory agent in neuroprotection studies against ischemia-reperfusion injury in rats,[95] and in the treatment of endometriosis in mice.[95, 96] However, oral bioavailability was extremely poor and it had a plasma half-life of less than half an hour.[97]

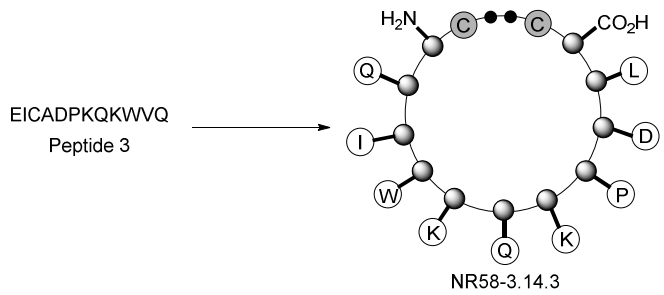


Figure 8: Peptide 3 (single letter amino acid code) and the retroinverse analogue, NR58-3.14.3. The shaded circles represent the carbons of the D-amino-acids, and the *C* and *N* terminals are marked. The peptide was oxidatively cyclised between the two terminal cysteine residues[98]

Structural-activity relationship analysis was performed on the original peptide 3 sequence, dividing it first into two hexapeptides, EICADP and KQKWVQ.[99] Both of these inhibited leukocyte migration, but only the *C*-terminal fragment retained the potency of the parent protein. This was further divided into two tripeptides, KQK and WVQ. WVQ inhibited all chemokines tested (MCP-1, MIP-1α, RANTES, IL8 and SDF-1α) with roughly equal potency to peptide 3. In contrast, the KQK tripeptide which also showed good MCP-1 inhibition *in vitro*, but displayed no broad spectrum chemokine inhibition and was not pursued further. Further abbreviation of the WVQ tripeptide destroyed the activity. Substitution studies identified a lipophilic tail group (tryptophan, tyrosine or phenylalanine) and a glutamine head group as key elements for retention of activity (Table 1). [99] The middle amino-acid could be any small aliphatic amino acid without impeding activity, though the glycine analogue (WGQ) was most active.

Peptide	ED_{50} vs. MCP-1 (μM)
EICADPKQKWVQ	8 ± 3
EICADP	50 ± 17
KQKWVQ	6 ± 3
KQK	12 ± 4
WVQ	4 ± 2

Table 1: The concentration of peptide 3 fragments required to reduce leukocyte migration by 50 % (ED_{50}). Migration of THP1 cells was induced with 25 ng/mL MCP-1 in the presence of a various concentrations of the peptides. The ED_{50} value is the mean ± sem of three separate measurements[99]

From this WxQ motif, a variety of non-peptide, low molecular weight acylglutamine and acylamino-glutarimides derivatives were synthesised. The most promising of these, NR58.4 (Figure 9), was effective in blocking leukocytes in LPS induced inflammation in addition to inhibiting chemokine-mediated chemotaxis with good potency, showing an ED_{50} of 5-15 nM *in vitro* (against MCP-1 induced migration of THP1 cells), and 0.3 mg kg^{-1} *in vivo* (against LPS-induced TNF-α migration in mice).[99] Despite being around four times as potent as peptide 3 in this model of inflammation, NR58.4 was inactive in models of chronic inflammation in which NR58-3.14.3 was known to be effective. This lack of potency was primarily due to the rapid enzymatic metabolism of the aminoglutarimide ring *in vivo*, eventually affording the biologically inactive glutamate derivate (Figure 9).[101]

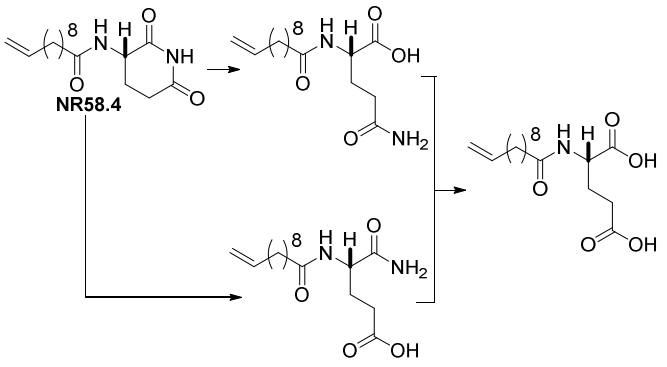


Figure 9: The probable metabolic fate of NR58.4[100]

In order to circumvent this, the 6-deoxo analogue of NR58.4 analogue, (Figure 10), was investigated as, despite displaying considerably lower BSCI activity (~100nM compared to 5 nM for NR58.4),[99] it was almost completely stable in serum. A range of 5 and 7-membered lactam analogues were synthesised in an attempt to improve the activity while retaining the stability, with a variety of alkyl chains. It had been previously noted that the (*R*) enantiomer of NR58.4 was at least 50 times less active than the (*S*) enantiomer, and this stereo-preference was found to be generally true for the lactam analogues.[100]

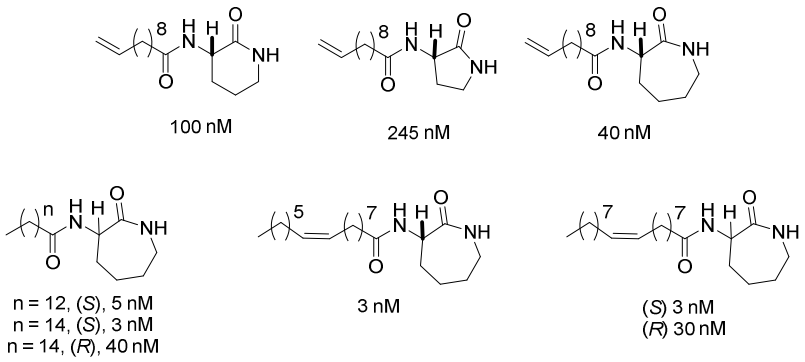


Figure 10: Lactam analogues of NR58.4, showing ED_{50} values for MCP-1 induced leukocyte migration *in vitro*[100]

The most active of these, a seven-membered lactam with an unsaturated 16-carbon tailgroup (Figure 10), had comparable BSCI activity to NR58.4 both *in vivo* and *in vitro*, but was significantly more stable in serum. However, the potency was not mirrored when it was administered orally, falling to an ED_{50} of 1mg/kg.[100] Further experimentation showed that including a quaternary substituent on the 2' position improved BSCI activity both *in vitro* and *in vivo*, with saturated chains giving more potent compounds than unsaturated. A series of increasingly truncated adamantane substituted compounds (Figure 11) had improved BSCI activity to the long chain compound when administered both orally and subcutaneously in the endotoxemia model of inflammation. In the case of the bulkier compounds, this did not translate into good pharmacokinetic profiles following single dose administration in rats. Investigations into the metabolic fate of these compounds showed both were rapidly

hydroxylated, presumably by cytochrome P450 enzymes in the liver. In contrast, the simple *tert*-butyl substituted lactam (Figure 11) showed a promising combination of activity, pharmacokinetic parameters and potency.[102] A similar compound to this, named FX125L, was chosen as the lead compound. FX125L started Phase 2 clinical trials, showing promise in the treatment of allergic asthma and other diseases related to inflammation.[103] Importantly, throughout the development of FX125L, no obvious unfavourable toxicological effects were noted.[100] Regulatory preclinical studies with FX125L suggested a very good safety profile and wide therapeutic range.[104] It was acquired by Boehringer Ingelheim for an undisclosed sum in July 2012.[105]

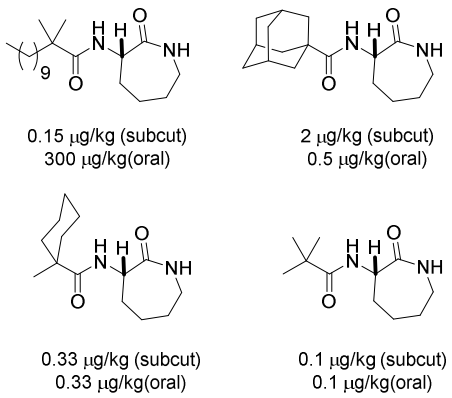


Figure 11: Subcutaneous and oral ED_{50} values for inhibition of LPS induced TNF-α model of inflammation *in vivo*[102]

1.6.1 NATURALLY OCCURRING LACTAMS

Naturally occurring 6- or 7-membered lactams are not common, but there exist a few which have been biosynthesised and offer potent biochemical effects. Bengamides and cilitamides (Figure 12) are both currently being investigated as potential sources of new drug candidates.[106, 107]

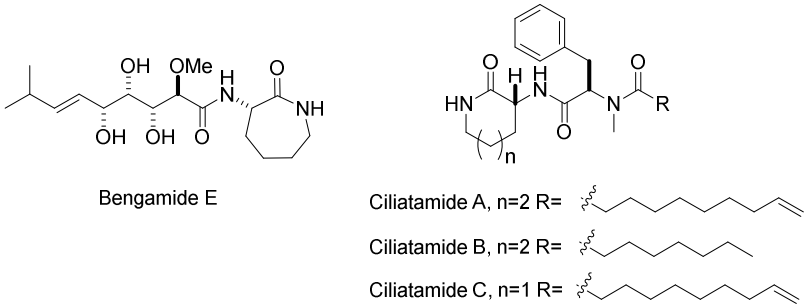


Figure 12: The structure of Bengamide E and three ciliatamides. Both molecules have well defined stereochemistry which affects their biological activity

Gramicidin S is a cyclic decapeptide composed of two identical dimers, Val-Orn-Leu-D-Phe-Pro.[108] The reaction sequence of gramicidin S synthetase consists of 16 steps, starting with a phenylalanine racemase (gramicidin S synthestase 1, GS1), followed by activation of the amino-acids by aminoacyl transferase and thioester formation.[109] These activated units are linked together by a series of transpeptidation and transthiolation reactions.[108] It was noted that in some circumstances side-reactions can occur, leading to early termination of the peptide chain.[110] The ornithine linked thioester intermediate is particulary unstable and, especially in the absence of L-leucine, this can form a *C*-terminal lactam tetrapeptide, D-Phe-Pro-Val-cyclo-Orn (Figure 13).[110, 111]

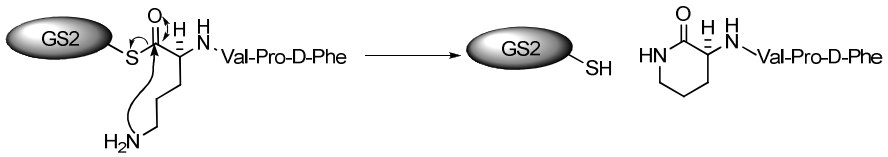


Figure 13: The possible early termination reaction at gramicidin S synthetase 2 (a condensing multi-enzyme) to give a *C*-terminal lactam peptide[110]

As well as occurring as a by-product from the natural gramicidin S synthase, this reaction can occur during solid phase synthesis. When synthesising the pentapeptide substrate analogue of gramicidin, Boc-D-Phe-Val-Pro-Orn-Leu-S-linker-TG, Bu *et al.* reported that, in the absence of the leucine residue, the cleaved *C*-terminal lactam peptide Boc-D-Phe-Val-Pro-cyclo-Orn was formed.[112] It seems likely that the δ-NH_2 of the deblocked ornithine cleaves the thioester to form the cyclised lactam.

1.6.2 Mode of Action of BSCIs

During the early stages of BSCI development, the mechanism by which the molecules inhibited chemokine migration was unclear. Biotinylated peptide 3 was found to bind to cells that expressed no chemokine receptors with the same affinity as to those in which chemokine receptors were expressed at over one million copies per cell. In addition, the binding of chemokines to receptors was found to be unaffected by the presence of peptide 3 and its derivatives.[113] Early structure-activity relationship studies showed that any modification to the molecules had the same effect against all chemokines tested. The possibility of BSCIs binding to the chemokine ligands themselves was also ruled out. Taken together, these facts suggested that the BSCIs were binding to a single, extracellular target,[113] showing a correlation between binding affinity at this site and potency of chemokine inhibition. These BSCI molecules do not block early cell signalling events linked to leukocyte migration, such as calcium mobilisation, nor the chemokine induced down-regulation of chemokine receptors, and it seems likely that BSCIs target a compenent of the intracellular signalling pathway related only to chemokine migration.[113]

Following extensive receptor screening, it was shown that both peptide and the current aminolactam BSCIs bind to the type 2 somatostatin receptor ($sstr_2$), an extracellular GPCR.[114, 115] For this reason, they are now known as somatotaxins (**somato**statin-mediated chemo**tax**is **in**hibitors).[114]

1.7 SOMATOSTATIN

Somatostatin is an endogenous regulatory peptide that is widely distributed throughout the central nervous system (CNS), the endocrine system, and the gastrointestinal tract.[116] It was first identified as a cyclic 14 amino-acid peptide (SST-14) in hypothalamic extracts that inhibited the secretion of growth hormone in 1973 (Figure 14).[117] In 1980, a second bioactive form of somatostatin was isolated, as an NH_2-terminally extended, 28 amino-acid (SST-28) peptide.

Both SST-14 and SST-28 are generated by the post-translational processing of a larger, 116 amino-acid, residue named pre-prosomatostatin.[118] Somatostatin is released from cells in the central and peripheral nervous system,[119] in the GI tract,[120] and from certain immune cells[121] in response to a range of chemical and molecular signals including hormones, cytokines and nutrients.[116]

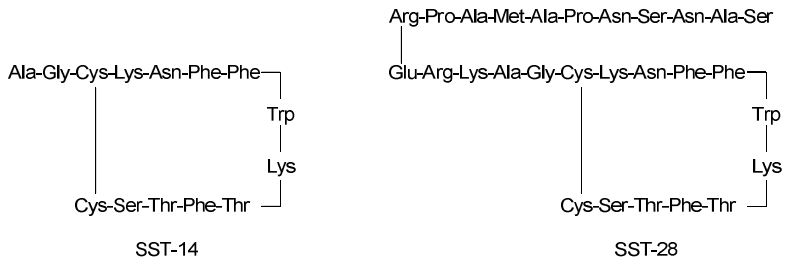


Figure 14: The primary structures of SST-14 and SST-28[122]

While initially noted for inhibition of growth hormone, somatostatin is now linked to the inhibition of a number of other hormones including thyrotropin and insulin, as well as neuro-enterohormones such as dopamine and exocrine secretions, and acts as a pan-inhibitory agent for all gastrointestinal hormones.[122, 123] Somatostatin can act as a neurotransmitter in the central nervous system, while in the peripheral nervous system it acts to downregulate neurogenic components of inflammation.[124, 125] In addition, somatostatin also has anti-proliferative effects in both normal dividing cells, as well as in abnormal tumours. These effects include both cytotoxic and cytostatic actions, and are mediated either directly by somatostatin receptors on the cell surface, or indirectly by somatostatin receptor on non-tumour cells. Binding of

somatostatin to these receptors leads to inhibition of the secretion of hormones and growth factors linked to tumour growth, as well as the inhibition of angiogenesis and mediation of immune cell function.[116, 126-129] Both SST-14 and SST-28 have extremely short half-lives (less than 3 minutes), so somatostatin producing cells and synaptic somatostatin stores are usually found near the target cells.[130]

Somatostatin exerts these, and other, physiological effects by binding to seven transmembrane domain (7TM) GPCRs found on the target cell surface. Five subtypes of somatostatin receptor, $sstr_{1\text{-}5}$, have been identified.[131, 132] These can be grouped into two subclasses based on ligand affinity and amino-acid identity, with $sstr_{2,3}$ and $sstr_5$ in one class, and $sstr_1$ and $sstr_4$ in the other. All five receptors bind SST-14 with a similar potency, but $sstr_5$ binds with a greater affinity to SST-28, and appears to be SST-28 selective.[133]

1.7.1 SOMATOSTATIN RECEPTORS

The somatostatin receptors are each encoded by separate genes on different chromosomes.[134] The gene encoding for $sstr_2$ contains an intron allowing the possibility of two isoforms, which differ in length of the cytoplasmic *C*-terminal.[135] $sstr_{2A}$, the longer isoform, is the most common and the only one expressed in humans, while $sstr_{2B}$ is the minor form.[136] Somatostatin receptors are expressed by a wide range of cells in varying concentrations, including those in the brain, pancreas, GI tract, liver and spleen.[136-138] In addition, $sstr_2$ and $sstr_3$ are known to be expressed by certain cells of the immune system including macrophages and T- and B- cells.[116] When activated by ligand binding, somatostatin receptors induce cellular responses by G-protein mediated messenger systems. These key second-messenger effects include the inhibition of adenylyl cyclase, activation of phosphotyrosine phospatase (PTP) and adjustment of mitogen-activated protein kinase (MAPK).[116] In addition, they activate several types of voltage channels in a subtype-specific fashion, including the stimulation of K^+ channels ($sstr_3$, $sstr_4$, $sstr_5$), activation of Ca^{2+} channels ($sstr_1$, $sstr_2$) and activation of an Na^+/H^+ exchanger ($sstr_1$).[139,

[140]Somatostatin receptors are known to be expressed on the surface of a number of tumours types, most commonly neuroendocrine tumours,[141] but also breast,[142] liver,[143] lung[144] and ovarian tumours[145] amongst others. Generally, tumour cells express more than one sstr subtype, with $sstr_2$ the most commonly expressed subtype. Subtypes 3 and 5 are also frequently expressed, but $sstr_4$ is uncommon.[146]

Due to the complex range of physiological effects elicited by the binding of somatostatin and associated ligands, it is difficult to tell whether each separate response is linked to only one sstr subtype, or is the result of multiple receptors working in concert.[116] However, some subtype selectivity has been identified. $sstr_3$ is known to elicit cytotoxic effects,[147] and $sstr_5$ appears to be unique among the sstr group in regulating insulin production.[148, 149] $sstr_2$ appears to be expressly involved in the inhibition of gastric acid secretion and immune responses. [150-153] Sstr2 is also expressed on the surface of a wide range of human and rat tumour cell lines, apparently in much greater quantities than other sstrs.[154, 155]

1.7.2 SOMATOSTATIN ANALOGUES

Somatostatin itself is unsuitable for clinical use due to rapid blood clearance and post-infusion hypersecretion of hormones,[156] and a number of analogues have been synthesised to overcome these problems. Based on the supposition that the sequence Phe_7-Trp-Lys-Thr_{10} was the essential pharmacophore fragment of somatostatin,[157, 158] the first analogue licensed for clinical use, octreotide, was reported in 1982 (Figure 15).[159]

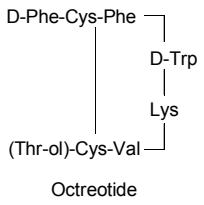


Figure 15: The structure of octreotide[160]

In monkeys, octreotide inhibited the release of growth hormone fortyfive times more powerfully than somatostatin, inhibits glucagon release eleven times more powerfully and insulin 1.3 times more powerfully.[161] Octreotide also showed far superior half-life, and showed no rebound hypersecretion compared to somatostatin.[162] However, unlike SST-14, octreotide was found to bind primarily to $sstr_2$, with lesser activity at the $sstr_3$ and $sstr_5$ subgroups.[163, 164] Octreotide (trade name Sandostatin®)[165] is approved for the treatment of a number of hormone secreting tumours, including growth hormone producing tumours and neuroendocrine tumours.[166, 167] Side effects associated with octreotide include cramps, diarrhoea, nausea and diabetic glucose tolerance.[168] In tests on $sstr_2^{-/-}$ and $sstr_2^{+/+}$ mice, binding of octreotide to $sstr_2$ was also shown to exhibit potent anti-inflammatory and anti-nociceptive activity.[169]

The development of somatostatin analogues and identification of their binding sites has provided a substantial amount of information about sstr subtypes and their modes of action. A number of somatostatin receptor subtype-specific analogues have been developed in an attempt to exploit possible subtype-specific physiological effects. These include BIM-23745 and BIM-23926 ($sstr_1$ specific), BIM-23197 and BIM-23120 ($sstr_2$ specific), L-796778 ($sstr_3$ specific) and BIM-23268 and BIM-23206 ($sstr_5$ specific), which were synthesised in an attempt to isolate the subtype associated with maximum anti-proliferation effects.[170] However, all but the $sstr_3$ subtype showed some anti-proliferative activity, and, as $sstr_3$ is known to hetereodimerise with other sstr subtypes, [171] it seems likely that the activation of all the sstr subtypes may be required to obtain maximal anti-proliferative effect.

TT-232 (Figure 16) binds predominately to $sstr_4$, with some activity at $sstr_1$. This compound had significant anti-inflammatory activity in a number of models both *in vivo* and *in vitro*,[124, 138] as well as showing anti-proliferative effects on tumour cells. [172, 173] Attempts to further probe sub-type selective responses using sstr knock-out mice have not clarified the situation. $Sstr_2$ has been convincingly linked to pituitary

GH secretion, but mice with a deficiency of $sstr_2$ develop normally both *in utero* and up to 15 months with the exception of a few minor side-effects, namely high levels of basal gastric acid and an absence of GH-induced feedback suppression of GH.[174]

Attempts to optimise the pharmacological response by developing multireceptor binding analogues have produced a number of peptide and non-peptide analogues of SST-14. KE-108 has a high affinity for all five sstr subtypes,[175] while SOM-230 binds with high affinity to $sstr_{1-3}$ and $sstr_5$ (Figure 16).[176] Despite being designed to mimic natural SST-14 binding and activity, both KE-108 and SOM-230 exhibit distinct signalling pathways to the natural ligand. In $sstr_2$ expressing cells, somatostatin was found to inhibit cAMP production, stimulated intracellular calcium and increased ERK phosphorylation.[177] Like somatostatin, both KE-108 and SOM-230 acted as full agonists for inhibition of adenylyl cyclase, but antagonised somatostatins effects on intracellular calcium and ERK phosphorylation. This implies that both KE-108 and SOM-230 exhibit functional selectivity (*vide infra*) different signalling pathways at $sstr_2$.[177]

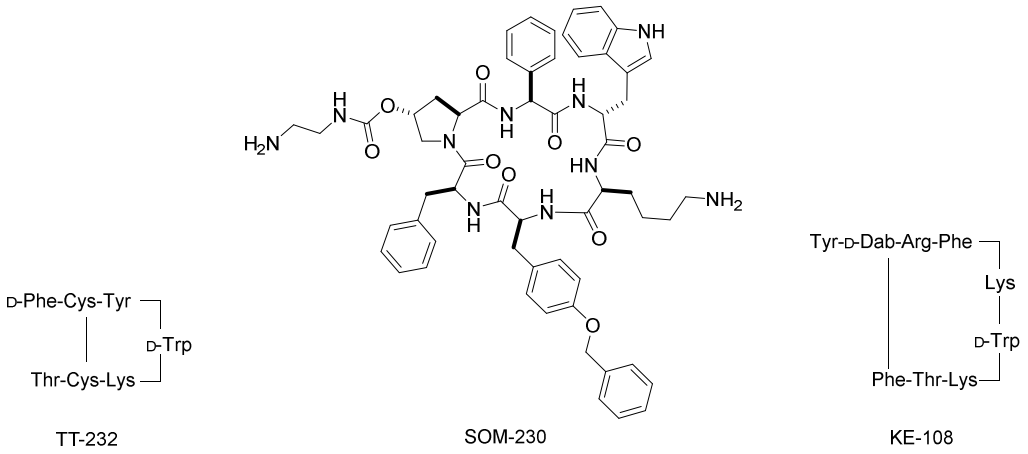


Figure 16: The structures of TT-232, KE-108 and SOM-230[173, 178]

1.7.3 INFLAMMATION AND SSTR$_2$

Though somatostatin is classically linked to hormone inhibition, it has been linked to inflammation for a number of years. Somatostatin is known to exhibit anti-proliferative effects on T lymphocytes and granulocytes,[179] as well as displaying

inhibitory activities on human natural killer cells, [180] and the release of inflammatory mediators such as TNF-α, IL-1β, and IL-6 cells IFN-γ.[136, 181-183] Somatostatin binding sites were first identified on human monocytes and lymphocytes in 1981,[184] and since then they have been identified on a variety of immune and haemopoietic cells.[185] Radio-labelling and fluorescent studies have shown the presence of sstrs on B and T lymphocytes from a variety of sites around the body, as well as thymocytes and human leukaemic cells.[181, 186-188] In addition, somatostatin is expressed by a range of inflammatory and immune cells including lymphocytes, macrophages, synovial, cells and thymocytes. [189],[152, 190]

$Sstr_2$ has been identified as the most common sstr subtype expressed by inflammatory cells,[136, 191] and as such has been studied extensively as a target for novel anti-inflammatory therapies. Both octreotide and a related compound, pasireotide, were shown to exhibit anti-inflammatory actions mediated primarily by the $sstr_2$ subtype.[169] It should be noted however, that $sstr_2$ is not the only sstr associated with anti-inflammatory activity. TT-232 is a cyclopenta-ring somatostatin analogue identified as a potent anti-inflammatory with no associated endocrine effects and binds exclusively to $sstr_4$, with some $sstr_1$ activity.[124, 192 138]

The essential sequence for binding of $sstr_2$ has been investigated using MK-678 (c[*N*-Me-Ala-Tyr-D-Trp-Lys-Val-Phe]), a somatostatin agonist that binds to potently to $sstr_2$, but not to $sstr_1$ or $sstr_3$.[193, 194] A series of $sstr_1$ and $sstr_2$ chimeras were tested to find the specific regions needed for MK-678 binding and this identified the residues Phe_{294}-Asp_{295}-Phe_{296}-Val_{297} in trans-membrane region 7 as the essential region for $sstr_2$ binding.

Work by Kaupmann *et al.* showed that a phenylalanine residue in the trans-membrane VII helice and an asparagine residue in trans-membrane VI were necessary for the binding of octreotide to human $sstr_2$, interacting with the Phe-Phe-Trp-Lys-Thr-Phe (amino-acids 6 to 11) sequence of SST-14 (Figure 17).[195]

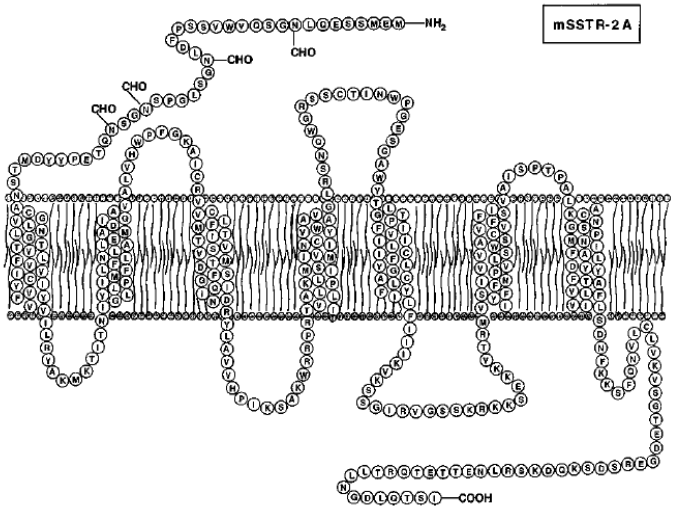


Figure 17: The structure of $sstr_2$ (taken from Reisine *et al.*)[196]

1.7.4. FUNCTIONAL SELECTIVITY

Functional selectivity posits that, rather than each sstr subtype educing a single discrete set of effects on agonist binding, instead different agonists may elicit different responses from the same receptor.[197] 'Traditional' receptor theory states that an agonist is a drug that activates a receptor, and this can either be a full agonist (maximally stimulate all effects linked to the receptor), a partial agonist (limited receptor response), a neutral antagonist (preventing activation of a receptor without eliciting a response) or an inverse agonist (reduce constitutive signalling).[198, 199] Therefore it would be expected that a full agonist would activate all signalling pathways to the same extent as the endogenous ligand. Equally, this theory suggests that a ligand that antagonizes one signal associated with a receptor should also antagonize all other signals effected by the receptor to the same extent.[199] However, this cannot explain how agonists such as KE 108 and SOM 230 are able to act as both agonists and antagonists for different somatostatin agonist pathways at $sstr_2$. The concept of functional selectivity was first described for nuclear receptors, but has also be described for a number of GPCRs.[200, 201]

Following the identification of $sstr_2$ as the binding site for the somatotaxin FX125L, a number of molecules analogous to both somatostatin and the early BSCIs were

synthesised to probe the relationship between 'classical' somatostatin activities e.g. growth hormone inhibition, and 'non-classical' BSCI activity, i.e. anti-inflammatory activity, at the $sstr_2$ subtype receptor.[202]

1.7.5 SOMATOTAXINS AND SSTR$_2$

As the Phe-Trp-Lys residues of SST-14 have found to be essential for bioactivity, it is not suprising that a large number of small molecule $sstr_2$ ligands have been developed, based the KWF motif. In particular, Merck has developed a wide range of structurally related compounds that have been shown to be potent $sstr_2$ agonists that inhibit growth hormone release *in vitro* (Figure 18).[203-205]

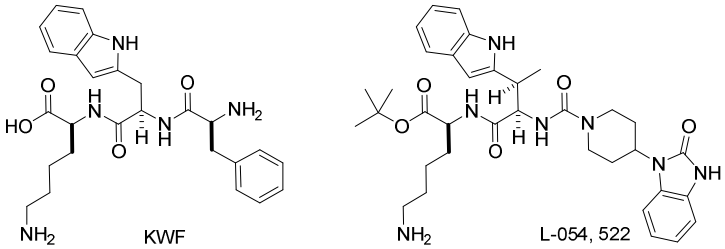


Figure 18: The KWF sequence, and the related $sstr_2$ ligand, L-054, 522[203]

The KWF motif overlaps with the tripeptide sequence WxQ from which the current families of somatotaxins are derived. The presence of the lysine preceding the WxQ is therefore potentially significant in explaining the binding of BSCIs to $sstr_2$.[99] The functional selectivity displayed by $sstr_2$ when these two structurally related families of compounds bind (growth hormone inhibition for KWF and anti-inflammatory activity for the somatotaxins) implied it should be possible to manipulate the physiological response (tip it more towards one effect than another) by synthesising a range of hybrid somatostatin/somatotaxin analogues. A variety of KWFQ, KWF, WF, WFQ and FQ analogues were synthesised to identify the critical motifs responsible for both $sstr_2$ binding and somatotaxin activity (Figure 19).[202]

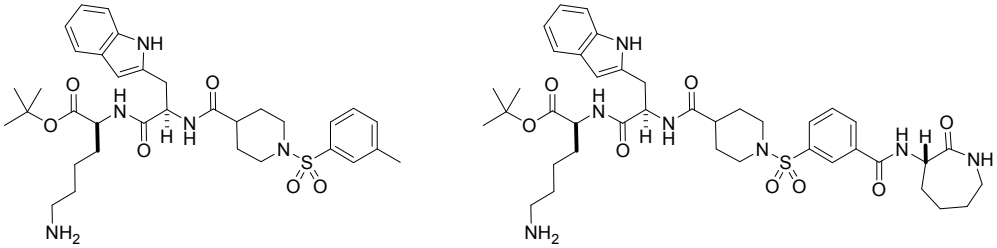


Figure 19: The structures of one of the KWF mimics (left) and one of the lactam containing KWFQ mimics (right)

Unsurprisingly, the KWF and KWFQ mimics showed the highest $sstr_2$ binding activities, judged by displacement of fluorescently labelled somatostatin (KWF = 97%, KWFQ = 98%; % inhibition of labelled SST14 at $sstr_2$ at 1 nm concentration). More interestingly, both of these sets of compounds also showed very high inhibition of neutrophil migration *in vitro*, with some of the KWF mimics up to 100-fold more potent than the KWFQ mimics (KWF = 95%, KWFQ = 115%, % inhibition of neutrophil migration at 1 nm concentration). This was surprising, as these results showed that the WxQ or lactam motifs are not necessary for BSCI activity.

For these larger compounds, there appeared to be a correlation between the binding activity of the ligands at $sstr_2$ (and hence the level of displacement of SST14), and the inhibition of neutrophil migration. However, the current small molecule somatotaxins produce a potent anti-inflammatory effect, with extremely low levels of somatostatin displacement (Figure 20).

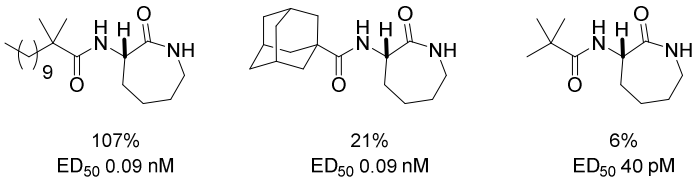


Figure 20: % somatostatin inhibition and leukocyte migration inhibition

The bulkier alkyl chain substituents appeared to compete more strongly with SST14 but this did not necessarily correspond with improved BSCI activity. This implies that BSCI activity is not, in fact, linked to the ability of the ligand to displace $sstr_2$ bound SST-14.

As the endogenous ligand for $sstr_2$, SST-14 would be expected to have the maximum binding activity However, though SST-14 is known to have anti-inflammatory properties it only exerted a maximum of 50 % inhibition of leukocyte migration.[202] The fact that the small molecule somatotaxins are able to exert an extremely potent anti-inflammatory effect without displacing SST-14 implies that they bind to a region of the receptor not inhabited by somatostatin. In addition to the orthosteric binding site, which binds the endogenous ligand, GPCRs are known to exhibit allosteric binding sites. These are topographically distinct binding sites, and have the potential to modulate or produce distinct physiological effects compared to the orthosteric site.[206-208] It seems likely that the somatotaxins bind to an allosteric site of $sstr_2$ close to the orthosteric site. This would explain why the smallest somatotaxins are able to bind without blocking somatostatin, while the bulkier somatotaxins both exert an allosteric effect and compete with somatostatin (Figure 21).

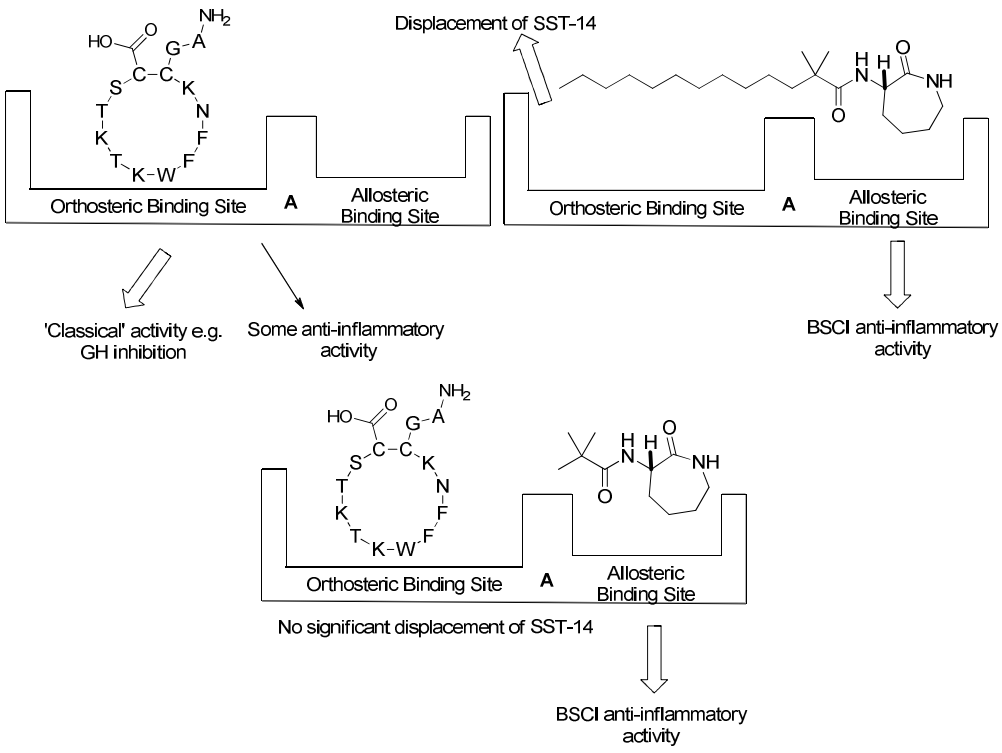


Figure 21: Model of binding of ligands to $sstr_2$ *via* proposed orthosteric and allosteric binding sites

It seems likely that binding to the orthosteric site of $sstr_2$ has the potential for some anti-inflammatory activity, as shown by the KWF and KWFQ mimics, while binding to the allosteric site provides the maximal BSCI activity.

As a natural $sstr_2$ mediated anti-inflammatory mechanism exists that is not maximally induced by SST-14, it seems likely that a non-somatostatin endogenous ligand must exist for this allosteric site that takes advantage of this. The fact that the small molecules somatotaxins, including FX125L, are amino-acid derived, extremely potent BSCIs and very well tolerated *in vivo* implies that they could be structurally related to these endogenous ligands.

Ornithine is not one of the proteinogenic amino-acids, as it is generally considered to be unsuitable for incorporation into stable peptides.[209] Ornithine rapidly cyclises in peptides and activated esters, resulting in lactam formation and scission of the amide/ester bond, even in extremely mild conditions.[210-212] Instead metabolism of arginine by arginase enzymes and L-arginine:glycine amidinotransferase is the main source of ornithine in the body.[213, 214] Interestingly, inflammation and infection leads to increased levels of arginine and hence elevated levels of ornithine.[215-217] Normally, ornithine is then metabolised further to polyamines and proline but potentially, excess ornithine could lead to greater ornithine incorporation into peptide precursors.

We propose that these endogenous ligands could be *C*-terminal lactam peptides, equivalent to the synthesised somatotaxins, formed during inflammatory episodes by cleavage of precursor peptide at lysine residues or at arginine residues with additional guanidine hydrolysis to create ornithine. This would provide a novel anti-inflammatory pathway *in vivo*. Equally, these CTLPs, if formed transiently and in low concentrations, may act as natural 'negative feedback' regulators *via* this allosteric, non-classical, $sstr_2$-mediated pathway. No conclusive evidence has yet been found for their existence, let alone the mechanism for an anti-inflammatory

response, but there is accumulating circumstantial evidence for their production within the body.

1.8 MECHANISMS OF ENDOGENOUS CTLP FORMATION

There are a number of mechanisms by which endogenous *C*-terminal lactam peptides could be synthesised from peptidyl lysine or arginine. Thrombin cleaves peptides including preprosomatostatin, fibrinogen and secretin at lysine residues with high specificity.[218] These could potentially form *C*-terminal 7-membered lactam peptides as a minor product during periods of peptidase enzyme activity, such as an inflammatory episode. The fact they have not been isolated is not necessarily proof of their non-existence, as it is likely they would occur in only very small amounts.

E. coli lysyl-tRNA synthase is known to edit ornithine by converting it into ornithine lactam by a pre-transfer mechanism, and it seems likely that this also occurs in the human body.[219] Due to the instability of ornithine in growing peptide chains, naturally occurring ornithine-containing peptides are rare, but a number have been identified in bacterium, primarily ornithine lipids extracted from both gram-negative and gram-positive bacteria (Figure 22).

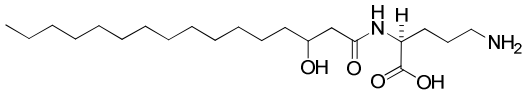


Figure 22: α-*N*-(3-Hydroxyhexadecanoyl)ornithine, a lyso-ornithine lipid produced by OlsB from *S. meliloti* strain 1021[220]

While ornithine lipids are quite widespread in bacteria,[221] only one ornithine containing peptide has been isolated from multicellular organism. This tripeptide, α-*N*-[α-*N*-(β-alanyl)-L-ornithyl]-L-ornithine, was isolated from the brackish-water bivalve *Corbicula japonica* but as yet little is known about its physiological role.[222]

In patients with the metabolic disorders, hyperornithinemia-hyperammonemia-homocytrullinemia (HHH) syndrome and gyrate atrophy of the choroid and retina,[223-225] unnaturally high levels of ornithine accumulate in the body. A

significant proportion of this is excreted in the urine as the ornithine lactam, apparently by spontaneous cyclisation of the free ornithine in the body.[226, 227] Significantly, low concentrations of the ornithine-lactam (average concentration 0.364 μM) are also known to be present in the plasma of healthy humans.[228] This suggests that there is an equilibrium between the open-chain and lactam forms of ornithine in the plasma.

In higher organisms, inteins could also provide a mechanism for the cleavage of peptides and formation of CTLPs. These are self-extracting peptide stretches that catalyse their own excision from a protein strand. This requires a specific amino acid sequence and involves an N-S/ N-O transfer from the oxygen of a serine or the sulphur of a cysteine residue binding to the *N*-extein, to effectively provide an activated ester or thioester.[229, 230] This is followed by trans-esterification and ring closure to produce the excised intein (Figure 23).[230] This mechanism is analogous to that observed in the early termination reaction of Gramicidin synthase.[110]

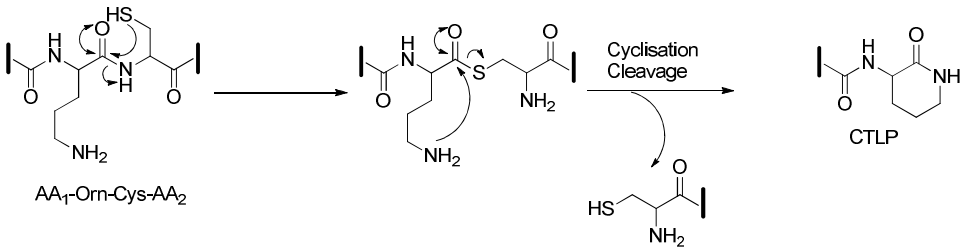


Figure 23: The hypothetical route to provide *C*-terminal 6-membered lactam peptides

1.9 DIRTY CHICKEN HYPOTHESIS

We posit that, in healthy individuals, a very small proportion of arginine or lysine-containing peptides may be converted to the equivalent CTLPs by one of the mechanisms proposed above, with production upregulated during times of inflammatory stress.

It has long been known that there is a link between heightened levels of inflammation and restricted growth. In 1963, Coates *et al.* described how chickens grew faster when kept in germfree conditions than those kept in the usual

environment.[231] First proposed by Solomons *et al.* in 1993, the 'Dirty Chicken Hypothesis' is based on this observation that chickens raised in unhygienic, overcrowded conditions tend to be smaller than those reared in similar conditions but given antibiotics which tend to grow to normal size.[232] Controlled studies showed that chicks reared in dirty conditions, as well as being smaller, showed high levels of interleukin 1, a protein that plays a major role in immune responses. This was not observed in either chicks raised in clean conditions, or those raised in dirty conditions but given antibiotics.[233] This analogy is used to explain the reason that children that grow up in squalid, impoverished conditions tend to be shorter than their wealthier counterparts, even if adequately fed. It is believed that constant exposure to infectious conditions causes a state of constant low-level inflammation, which in turn affects growth, believed to be caused by diversion of nutrients towards fighting infections, and poor uptake on nutrients due to inflammation of the gut.[234]

It would be expected that, unlike the small molecule anti-inflammatory lactams (e.g. *tert*-butyl substituted seven membered lactam, Figure 11), CTLPs could be larger and act as inhibitors of somatostatin binding to $sstr_2$ (cf. Figure 21). In healthy individuals, where CTLPs are present as part of the natural immune system, somatostatin binding is inhibited and normal growth *via* the production of GH is observed. In individuals with chronic inflammation and an exhausted pool of CTLP precursor proteins, CTLP levels will be low, resulting in little inhibition of somatostatin binding to $sstr_2$ and a resulting inhibition of GH production, and hence restricted growth.

1.10 RESEARCH AIMS AT START OF PROJECT

The primary aim of this part of the thesis was the synthesis of a range of peptidyl CTLPs that could be tested for their somatotaxin activity. A secondary aim was to use these compounds to provide more information about the endogenous CTLPs we postulate may be formed *in vivo*.

Initially, the synthesis of ten CTLPs was planned (Figure 24). These fell into three basic groups:

1) **15 atom tailgroups:** Three compounds, a di-, tri- and tetrapeptide, were synthesised with varying lengths of alkyl tail group (C11, C8 and C5 respectively) to give compounds with a similar structure, but different polarities, to assess how this would affect $sstr_2$ binding and somatotaxin activity.
2) **Acetylated CTLPs:** Three compounds were synthesised, a di-, tri and tetra-peptide, with an *N*-terminal acyl group to assess how peptide length might affect binding and somatotaxin activity.
3) **Boc-protected CTLPs:** The Boc-protected di-, tri-, tetra- and pentapeptides were synthesised both as potential novel somatotaxins or as tools for further investigation of the $sstr_2$ mediated anti-inflammatory mechanism.

1.11 REFERENCES

1. L. Ferrero-Miliani, O. H. Nielsen, P. S. Andersen and S. E. Girardin, *Clin. Exp. Immunol.*, 2007, **147**, 227-235.
2. S. Akira, S. Uematsu and O. Takeuchi, *Cell*, 2006, **124**, 783-801.
3. D. Tang, R. Kang, C. B. Coyne, H. J. Zeh and M. T. Lotze, *Immunol. Rev.*, 2012, **249**, 158-175.
4. D. V. Krysko, P. Agostinis, O. Krysko, A. D. Garg, C. Bachert, B. N. Lambrecht and P. Vandenabeele, *Trends Immunol.*, 2011, **32**, 157-164.
5. C. S. Calfee and M. A. Matthay, *Nature*, 2010, **464**, 41-42.
6. K. H. G. Mills, *Nat. Rev. Immunol.*, 2011, **11**, 807-822.
7. O. Takeuchi and S. Akira, *Cell*, 2010, **140**, 805-820.
8. T. H. Mogensen, *Clin. Microbiol. Rev.*, 2009, **22**, 240-273.
9. R. Medzhitov, *Nature*, 2008, **454**, 428-435.
10. K. Ebnet and D. Vestweber, *Histochem. Cell Biol.*, 1999, **112**, 1-23.
11. J. Middleton, A. M. Patterson, L. Gardner, C. Schmutz and B. A. Ashton, *Blood*, 2002, **100**, 3853-3860.
12. R. Yadav, K. Y. Larbi, R. E. Young and S. Nourshargh, *Thromb. Haemostasis*, 2003, **90**, 598-606.
13. E. J. Kunkel and E. C. Butcher, *Nat. Rev. Immunol.*, 2003, **3**, 822-829.
14. G. L. Larsen and P. M. Henson, *Annu. Rev. Immunol.*, 1983, **1**, 335-359.
15. J. R. Didsbury, R. J. Uhing and R. Snyderman, in *Mononuclear Phagocytes*, ed. R. Furth, Springer Netherlands, 1992, pp. 413-424.
16. T. A. Springer, *Cell*, 1994, **76**, 301-314.
17. H. L. Wright, R. J. Moots, R. C. Bucknall and S. W. Edwards, *Rheumatology*, 2010, **49**, 1618-1631.
18. C. Akgul, D. A. Moulding and S. W. Edwards, *FEBS Lett.*, 2001, **487**, 318-322.

19. J. S. Savill, A. H. Wyllie, J. E. Henson, M. J. Walport, P. M. Henson and C. Haslett, *J. Clin. Invest.*, 1989, **83**, 865-875.

20. R. Osuský, P. Malik and S. J. Ryan, *Ophthalmic Res.*, 1997, **29**, 31-36.

21. L. M. Coussens and Z. Werb, *Nature*, 2002, **420**, 860-867.

22. T. P. Janeway CA Jr, Walport M, et al. , in *Immunobiology: The Immune System in Health and Disease. 5th edition.*, Garland Science, New York, 2001.

23. B. Z. Schmidt and H. R. Colten, *Immunol. Rev.*, 2000, **178**, 166-176.

24. P. Davies, P. J. Bailey, M. M. Goldenberg and A. W. Ford-Hutchinson, *Annu. Rev. Immunol.*, 1984, **2**, 335-357.

25. E. Ricciotti and G. A. FitzGerald, *Arterioscler., Thromb., Vasc. Biol.*, 2011, **31**, 986-1000.

26. C. A. Dinarello, *CHEST Journal*, 2000, **118**, 503-508.

27. J. R. Bradley, *J. Pathol.*, 2008, **214**, 149-160.

28. S. Akira, T. Hirano, T. Taga and T. Kishimoto, *The FASEB Journal*, 1990, **4**, 2860-2867.

29. T. Kishimoto, *Blood*, 1989, **74**, 1-10.

30. J. M. Munro, J. S. Pober and R. S. Cotran, *Am. J. Pathol.*, 1989, **135**, 121.

31. J. Saklatvala, W. Davis, F. Guesdon, M. Karin and C. J. Marshall, *Philos. Trans. R. Soc., B*, 1996, **351**, 151-157.

32. J. M. Rodriguez-Frade, M. Mellado and C. Martinez-A, in *Cytokine Reference*, ed. J. a. F. Oppenheim, Marc, Academic Press, 2000, pp. 2041-2053.

33. L. C. Borish and J. W. Steinke, *J. Allergy Clin. Immunol.*, 2003, **111**, S460-S475.

34. P. C. Rummel, S. Thiele, L. S. Hansen, T. P. Petersen, A. H. Sparre-Ulrich, T. Ulven and M. M. Rosenkilde, *Mol. Pharmacol.*, 2013, **84**, 335-345.

35. A. Zlotnik, O. Yoshie and H. Nomiyama, *Genome Biol.*, 2006, **7**, 1-11.

36. S. J. Allen, S. E. Crown and T. M. Handel, *Annu. Rev. Immunol.*, 2007, **25**, 787-820.

37. M. Baggiolini and C. A. Dahinden, *Immunol. Today*, 1994, **15**, 127-133.

38. J. Van Damme, P. Proost, W. Put, S. Arens, J. Lenaerts, R. Conings, G. Opdenakker, H. Heremans and A. Billiau, *J. Immunol.*, 1994, **152**, 5495-5502.

39. P. M. Murphy, M. Baggiolini, I. F. Charo, C. A. Hébert, R. Horuk, K. Matsushima, L. H. Miller, J. J. Oppenheim and C. A. Power, *Pharmacol. Rev.*, 2000, **52**, 145-176.

40. R. Horuk and H. P. Ng, *Med. Res. Rev.*, 2000, **20**, 155-168.

41. A. Jatakanon, C. Uasuf, W. Maziak, S. A. M. Lim, K. F. Chung and P. J. Barnes, *Am. J. Respir. Crit. Care Med.*, 1999, **160**, 1532-1539.

42. E. H. S. Choy and G. S. Panayi, *N. Engl. J. Med.*, 2001, **344**, 907-916.

43. M. Locati, Massimo and M. Murphy, Philip M., *Annu. Rev. Med.*, 1999, **50**, 425-440.

44. G. K. Hansson, *N. Engl. J. Med.*, 2005, **352**, 1685-1695.

45. B. H. Maskrey, I. L. Megson, P. D. Whitfield and A. G. Rossi, *Arterioscler., Thromb., Vasc. Biol.*, 2011, **31**, 1001-1006.

46. R. G. Kurumbail, J. R. Kiefer and L. J. Marnett, *Curr. Opin. Struct. Biol.*, 2001, **11**, 752-760.

47. G. Dannhardt and W. Kiefer, *Eur. J. Med. Chem.*, 2001, **36**, 109-126.

48. A. A. Shah, D. J. Fitzgerald and F. E. Murray, *Ir. J. Med. Sc.*, 1999, **168**, 242-245.

49. G. F. Fabiola, V. Pattabhi and K. Nagarajan, *Bioorg. Med. Chem.*, 1998, **6**, 2337-2344.

50. R. S. Bresalier, R. S. Sandler, H. Quan, J. A. Bolognese, B. Oxenius, K. Horgan, C. Lines, R. Riddell, D. Morton, A. Lanas, M. A. Konstam and J. A. Baron, *N. Engl. J. Med.*, 2005, **352**, 1092-1102.

51. T. Trapp and F. Holsboer, *Trends Pharmacol. Sci.*, 1996, **17**, 145-149.

52. P. J. Barnes, *Eur. J. Pharmacol.*, 2006, **533**, 2-14.

53. N. Z. Lu, S. E. Wardell, K. L. Burnstein, D. Defranco, P. J. Fuller, V. Giguere, R. B. Hochberg, L. McKay, J.-M. Renoir, N. L. Weigel, E. M. Wilson, D. P. McDonnell and J. A. Cidlowski, *Pharmacol. Rev.*, 2006, **58**, 782-797.

54. T. Rhen and J. A. Cidlowski, *N. Engl. J. Med.*, 2005, **353**, 1711-1723.

55. H. S. Nelson, *J. Allergy Clin. Immunol.*, 2001, **107**, 397-416.

56. http://www.drugs.com/stats/top100/sales, Accessed February 05, 2014.

57. S. R. Salpeter, N. S. Buckley, T. M. Ormiston and E. E. Salpeter, *Ann. Intern. Med.*, 2006, **144**, 904-912.

58. T. Y. Shen, in *A Textbook of Drug Discovery and Development*, eds. P. Krogsgaard-Larsen and H. Bundgaard, Harwood Academic Press, 2 edn., 1992, pp. 457-458.

59. H. Schäcke, W.-D. Döcke and K. Asadullah, *Pharmacol. Ther.*, 2002, **96**, 23-43.

60. P. J. Barnes and I. M. Adcock, *Lancet*, **373**, 1905-1917.

61. C. R. Mackay, *Nat. Immunol.*, 2001, **2**, 95-101.

62. A. D. Luster, R. Alon and U. H. von Andrian, *Nat. Immunol.*, 2005, **6**, 1182-1190.

63. C. R. Mackay, *Nat. Immunol.*, 2008, **9**, 988-998.

64. Z. Johnson, C. A. Power, C. Weiss, F. Rintelen, H. Ji, T. Ruckle, M. Camps, T. N. C. Wells, M. K. Schwarz, A. E. I. Proudfoot and C. Rommel, *Biochem. Soc. Trans.*, 2004, **32**, 366-377.

65. A. E. Koch, *Arthritis Rheum.*, 2005, **52**, 710-721.

66. R. M. Strieter, M. D. Burdick, B. N. Gomperts, J. A. Belperio and M. P. Keane, *Cytokine Growth Factor Rev.*, 2005, **16**, 593-609.

67. C. A. Haskell, S. Ribeiro and R. Horuk, *Curr. Opin. Investig. Drugs*, 2002, **3**, 399-405.

68. P. Lusso, *Vaccine*, 2002, **20**, 1964-1967.

69. M. Westby and E. van der Ryst, *Antivir. Chem. Chemother.*, 2005, **16**, 339-354.

70. S. Abel, D. J. Back and M. Vourvahis, *Antivir. Ther.*, 2009, **14**, 607-618.

71. J. M. Strizki, C. Tremblay, S. Xu, L. Wojcik, N. Wagner, W. Gonsiorek, R. W. Hipkin, C.-C. Chou, C. Pugliese-Sivo, Y. Xiao, J. R. Tagat, K. Cox, T. Priestley, S. Sorota, W. Huang, M. Hirsch, G. R. Reyes and B. M. Baroudy, *Antimicrob. Agents Chemother.*, 2005, **49**, 4911-4919.

72. P. Yeni, A. Lamarca, D. Berger, P. Cimoch, A. Lazzarin, P. Salvato, F. M. Smaill, E. Teofilo, S. J. Madison, W. G. Nichols, K. K. Adkison, T. Bonny, J. Millard and D. McCarty, *HIV Med.*, 2009, **10**, 116-124.

73. W. G. Nichols, H. M. Steel, T. Bonny, K. Adkison, L. Curtis, J. Millard, K. Kabeya and N. Clumeck, *Antimicrob. Agents Chemother.*, 2008, **52**, 858-865.

74. T. Horn, *Vicriviroc Falls Short in Treatment-Experienced HIV Studies*, http://www.aidsmeds.com/articles/hiv_vicriviroc_ccr5_2446_18031.shtml, Accessed February 20th, 2014.

75. *Europe gives final approval to Pfizer HIV drug*, http://www.reuters.com/article/2007/09/24/pfizer-hiv-idUSL2354082520070924.

76. R. Reshef, S. M. Luger, E. O. Hexner, A. W. Loren, N. V. Frey, S. D. Nasta, S. C. Goldstein, E. A. Stadtmauer, J. Smith, S. Bailey, R. Mick, D. F. Heitjan, S. G. Emerson, J. A. Hoxie, R. H. Vonderheide and D. L. Porter, *N. Engl. J. Med.*, 2012, **367**, 135-145.

77. L. J. Wilson and D. C. Liotta, *Drug Dev. Res.*, 2011, **72**, 598-602.

78. E. De Clercq, *Pharmacol. Ther.*, 2010, **128**, 509-518.

79. J. A. Burger and D. J. Stewart, *Expert Opin. Invest. Drugs*, 2009, **18**, 481-490.

80. B. A. Zabel, W. W. Agace, J. J. Campbell, H. M. Heath, D. Parent, A. I. Roberts, E. C. Ebert, N. Kassam, S. Qin, M. Zovko, G. J. LaRosa, L. L.

Yang, D. Soler, E. C. Butcher, P. D. Ponath, C. M. Parker and D. P. Andrew, *J. Exp. Med.*, 1999, **190**, 1241-1256.

81. A. Zaballos, J. Gutiérrez, R. Varona, C. Ardavín and G. Márquez, *J. Immunol.*, 1999, **162**, 5671-5675.

82. M. J. Walters, Y. Wang, N. Lai, T. Baumgart, B. N. Zhao, D. J. Dairaghi, P. Bekker, L. S. Ertl, M. E. T. Penfold, J. C. Jaen, S. Keshav, E. Wendt, A. Pennell, S. Ungashe, Z. Wei, J. J. K. Wright and T. J. Schall, *J. Pharmacol. Exp. Ther.*, 2010, **335**, 61-69.

83. M.-A. Wurbel, M. G. McIntire, P. Dwyer and E. Fiebiger, *PLoS One*, 2011, **6**, e16442.

84. B. Eksteen and D. H. Adams, *IDrugs*, 2010, **13**, 472-781.

85. S. Keshav, T. Vaňásek, Y. Niv, R. Petryka, S. Howaldt, M. Bafutto, I. Rácz, D. Hetzel, O. H. Nielsen and S. Vermeire, *PLoS One*, 2013, **8**, e60094.

86. J. J. Haringman, D. M. Gerlag, T. J. M. Smeets, D. Baeten, F. van den Bosch, B. Bresnihan, F. C. Breedveld, H. J. Dinant, F. Legay, H. Gram, P. Loetscher, R. Schmouder, T. Woodworth and P. P. Tak, *Arthritis Rheum.*, 2006, **54**, 2387-2392.

87. C. E. Vergunst, D. M. Gerlag, L. Lopatinskaya, L. Klareskog, M. Smith, F. Van den Bosch, H. J. Dinant, Y. Lee, T. Wyant and E. W. Jacobson, *Arthritis Rheum.*, 2008, **58**, 1931-1939.

88. C. E. Vergunst, D. M. Gerlag, L. von Moltke, M. Karol, T. Wyant, X. Chi, E. Matzkin, T. Leach and P. P. Tak, *Arthritis Rheum.*, 2009, **60**, 3572-3581.

89. R. C. Russo, C. C. Garcia and M. M. Teixeira, *Curr. Opin. Drug Discov. Devel.*, 2010, **13**, 414-427.

90. J. Reckless and D. J. Grainger, *Biochem. J.*, 1999, **340**, 803-811.

91. A. Yadav, V. Saini and S. Arora, *Clin. Chim. Acta*, 2010, **411**, 1570-1579.

92. S. J. Myers, L. M. Wong and I. F. Charo, *J. Biol. Chem.*, 1995, **270**, 5786-5792.

93. V. L. Schweickart, A. Epp, C. J. Raport and P. W. Gray, *J. Biol. Chem.*, 2000, **275**, 9550-9556.

94. B. J. Rollins, *Blood*, 1997, **90**, 909-928.

95. J. S. Beech, J. Reckless, D. E. Mosedale, D. J. Grainger, S. C. R. Williams and D. K. Menon, *J. Cereb. Blood Flow Metab.*, 2001, **21**, 683-689.

96. U. A. Kayisli, M. Berkkanoglu, Lufang Zhang, G. Kizilay and A. Arici, *Reprod. Sci.*, 2007, **14**, 825-835.

97. S. M. Wilbert, G. Engrissei, E. K. G. Yau, David J., L. Tatalick and D. B. Axworthy, *Anal. Biochem.*, 2000, **278**, 14-21.

98. J. Reckless, L. M. Tatalick and D. J. Grainger, *Immunology*, 2001, **103**, 244-254.

99. D. J. Fox, J. Reckless, S. G. Warren and D. J. Grainger, *J. Med. Chem.*, 2002, **45**, 360-370.

100. D. J. Fox, J. Reckless, S. M. Wilbert, I. Greig, S. Warren and D. J. Grainger, *J. Med. Chem.*, 2005, **48**, 867-874.

101. D. J. Grainger, J. Reckless and D. J. Fox, *Mini-Rev. Med. Chem.*, 2005, **5**, 825-832.

102. D. J. Fox, J. Reckless, H. Lingard, S. Warren and D. J. Grainger, *J. Med. Chem.*, 2009, **52**, 3591-3595.

103. D. Grainger, J. Reckless, V. Piercy, K. Langley, I. Purvis and D. Fox, *Allergy*, 2009, **64**, 1418.

104. P. C. Wiesel, R.; Wang-Smith, L.; Reckless, J.;Champion, K. J.; Petrolese, V.;& Grainger, D, in *ACAAI's 2009 Annual Meeting,*, Miami Beach, Florida (USA), 2009.

105. *Boehringer Ingelheim and Funxional Therapeutics announce acquisition of Funxional's FX125L and the somatotaxin portfolio to treat inflammation*, http://www.boehringer-ingelheim.com/news/news_releases/press_releases/2012/23_july_2012_fxt.html, Accessed February 27 2014.

106. M. G. Banwell and K. J. McRae, *J. Org. Chem.*, 2001, **66**, 6768-6774.

107. J. A. Lewis, R. N. Daniels and L. C. W., in *Org. Lett.*, 2008, vol. 10, pp. 4545-4548.

108. A. Gadow, J. Vater, W. Schlumbohm, Z. Palacz, J. Salnikow and H. Kleinkauf, *Eur. J. Biochem.*, 1983, **132**, 229-234.

109. H. von Döhren, U. Keller, J. Vater and R. Zocher, *Chem. Rev. (Washington, DC, U. S.)*, 1997, **97**, 2675-2706.

110. J. Vater, W. Schlumbohm, Z. Palacz, J. Salnikow, A. Gadow and H. Kleinkauf, *Eur. J. Biochem.*, 1987, **163**, 297-302.

111. H. Holm, L. O. Frøholm and S. Laland, *Biochim. Biophys. Acta, Gen. Subj.*, 1966, **115**, 361-370.

112. X. Bu, G. Xie, C. W. Law and Z. Guo, *Tetrahedron Lett.*, 2002, **43**, 2419-2422.

113. D. J. Grainger and J. Reckless, *Biochem. Pharmacol.*, 2003, **65**, 1027-1034.

114. D. J. Grainger, WO/2010/097600, 2010.

115. E. Konstantinos, R. Jill, O.-S. Catherine, P. Val, P. Ian, F. David, W. Dan and G. David, in *Airway Inflammation: From Mechanisms to Non-Invasive Biomarkers*, American Thoracic Society, 2011, pp. A4460-A4460.

116. Y. C. Patel, *Front. Neuroendocrinol.*, 1999, **20**, 157-198.

117. P. Brazeau, W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier and R. Guillemin, *Science*, 1973, **179**, 77-79.

118. T. G. Warren and D. Shields, *Cell*, 1984, **39**, 547-555.

119. M. Schindler, P. P. A. Humphrey and P. C. Emson, *Prog. Neurobiol.*, 1996, **50**, 9-47.

120. C. H. S. McIntosh, *Life Sci.*, 1985, **37**, 2043-2058.

121. A. M. ten Bokum, L. J. Hofland and P. M. van Hagen, *Eur. Cytokine Netw.*, 2000, **11**, 161-176.

122. M. Pawlikowski, *Physiology of Somatostatin*, Austin, TX: Landes Bioscience, 2007.

123. S. Reichlin, *J. Lab. Clin. Med.*, 1987, **109**, 320-326.

124. E. Pintér, Z. Helyes and J. Szolcsányi, *Pharmacol. Ther.*, 2006, **112**, 440-456.

125. T. Reisine and G. I. Bell, *Neuroscience*, 1995, **67**, 777-790.

126. M. C. Pagliacca, R. Tognellini, F. Grignani and I. Nicoletti, *Endocrinology*, 1991, **129**, 2555-2562.

127. K. Sharma, Y. C. Patel and C. B. Srikant, *Mol. Endocrinol.*, 1996, **10**, 1688-1696.

128. K. Sharma and C. B. Srikant, *Int. J. Cancer*, 1998, **76**, 259-266.

129. K. Sharma and C. B. Srikant, *Biochem. Biophys. Res. Commun.*, 1998, **242**, 134-140.

130. I. Modlin, M. Pavel, M. Kidd and B. Gustafsson, *Aliment. Pharmacol. Ther.*, 2010, **31**, 169-188.

131. Y. Yamada, S. R. Post, K. Wang, H. S. Tager, G. I. Bell and S. Seino, *Proc. Natl. Acad. Sci.*, 1992, **89**, 251-255.

132. J. F. Bruno, Y. Xu, J. Song and M. Berelowitz, *Proc. Natl. Acad. Sci.*, 1992, **89**, 11151-11155.

133. Y. C. Patel, M. Greenwood, R. Panetta, N. Hukovic, S. Grigorakis, L.-A. Robertson and C. B. Srikant, *Metabolism*, 1996, **45**, 31-38.

134. G. Olias, C. Viollet, H. Kusserow, J. Epelbaum and W. Meyerhof, *J. Neurochem.*, 2004, **89**, 1057-1091.

135. Y. C. Patel, M. Greenwood, G. Kent, R. Panetta and C. B. Srikant, *Biochem. Biophys. Res. Commun.*, 1993, **192**, 288-294.

136. D. E. Elliott, J. Li, A. M. Blum, A. Metwali, Y. C. Patel and J. V. Weinstock, *Eur. J. Immunol.*, 1999, **29**, 2454-2463.

137. M. Schindler, P. P. Humphrey and P. C. Emson, *Prog. Neurobiol.*, 1996, **50**, 9-47.

138. E. Pintér, Z. Helyes, J. Németh and J. Szolcsányi, in *NeuroImmune Biology*, ed. J. Gábor, Elsevier, 2009, vol. 8, pp. 119, 121-134.

139. Z. Csaba and P. Dournaud, *Neuropeptides*, 2001, **35**, 1-23.

140. S. Krantic, I. Goddard, A. Saveanu, N. Giannetti, J. Fombonne, A. Cardoso, P. Jaquet and A. Enjalbert, *Eur. J. Endocrinol.*, 2004, **151**, 643-655.

141. M. Volante, R. Rosas, E. Allìa, R. Granata, A. Baragli, G. Muccioli and M. Papotti, *Mol. Cell. Endocrinol.*, 2008, **286**, 219-229.

142. H. L. Watt, G. Kharmate and U. Kumar, *Mol. Cell. Endocrinol.*, 2008, **286**, 251-261.

143. D. Dimitroulopoulos, D. Xinopoulos, K. Tsamakidis, A. Zisimopoulos, E. Andriotis, S. Markidou, D. Panagiotakos, C. Chrysohoou, A. Bazinis and E. Paraskevas, *Hepato-Gastroenterology*, 2002, **49**, 1245-1250.

144. L. Righi, M. Volante, V. Tavaglione, A. Billè, L. Daniele, T. Angusti, F. Inzani, G. Pelosi, G. Rindi and M. Papotti, *Ann. Oncol.*, 2010, **21**, 548-555.

145. G. Hall, L. Turnbull, I. Richmond, L. Helboe and S. Atkin, *Br. J. Cancer*, 2002, **87**, 86-90.

146. L.-C. Sun and D. H Coy, *Curr. Drug Delivery*, 2011, **8**, 2-10.

147. I. Minsel, R. Mentlein, S. Sel, Y. Diebold, L. Bräuer, E. Mühlbauer and F. P. Paulsen, *Endocrinology*, 2009, **150**, 2254-2263.

148. T. Tirone, M. Norman, S. Moldovan, F. DeMayo, X. Wang and F. Brunicardi, *Pancreas*, 2003, **26**, e67-e73.

149. S. W. Mitra, É. Mezey, B. Hunyady, L. Chamberlain, E. Hayes, F. Foor, Y. Wang, A. Schonbrunn and J. M. Schaeffer, *Endocrinology*, 1999, **140**, 3790-3796.

150. V. Martinez, A. P. Curi, B. Torkian, J. M. Schaeffer, H. A. Wilkinson, J. H. Walsh and Y. Taché, *Gastroenterology*, 1998, **114**, 1125-1132.

151. L. Piqueras, Y. Taché and V. Martínez, *J. Physiol.* 2003, **549**, 889-901.

152. D. E. Elliott, A. M. Blum, J. Li, A. Metwali and J. V. Weinstock, *J. Immunol.*, 1998, **160**, 3997-4003.

153. Y. Takeba, N. Suzuki, M. Takeno, T. Asai, S. Tsuboi, T. Hoshino and T. Sakane, *Arthritis Rheum.*, 1997, **40**, 2128-2138.

154. P. A. Eden and J. E. Taylor, *Life Sci.*, 1993, **53**, 85-90.

155. J. E. Taylor, M. A. Theveniau, R. Bashirzadeh, T. Reisine and P. A. Eden, *Peptides*, 1994, **15**, 1229-1236.

156. W. De Herder, A. Van der Lely and S. Lamberts, *Postgrad. Med. J.*, 1996, **72**, 403-408.

157. D. F. Veber, F. W. Holly, R. F. Nutt, S. J. Bergstrand, S. F. Brady, R. Hisrschmann, M. S. Glitzer and R. Saperstein, *Nature*, 1979, **280**, 512-514.

158. D. F. Veber, R. M. Freidinger, D. S. Perlow, W. J. Paleveda, F. W. Holly, R. G. Strachan, R. F. Nutt, B. H. Arison, C. Homnick, W. C. Randall, M. S. Glitzer, R. Saperstein and R. Hirschmann, *Nature*, 1981, **292**, 55-58.

159. W. Bauer, U. Briner, W. Doepfner, R. Haller, R. Huguenin, P. Marbach, T. J. Petcher and J. Pless, *Life Sci.*, 1982, **31**, 1133-1140.

160. T. Talme and K.-G. Sundqvist, in *Nerve-Driven Immunity*, ed. M. Levite, Springer Vienna, 2012, pp. 203-225.

161. S. W. J. Lamberts, A.-J. van der Lely, W. W. de Herder and L. J. Hofland, *N. Engl. J. Med.*, 1996, **334**, 246-254.

162. S. W. Lamberts, R. Oosterom, M. Neufeld and E. D. Pozo, *J. Clin. Endocrinol. Metab.*, 1985, **60**, 1161-1165.

163. L. J. Hofland, H. A. Visser-Wisselaar and S. W. Lamberts, *Biochem. Pharmacol.*, 1995, **50**, 287-297.

164. S. Siehler, K. Seuwen and D. Hoyer, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 1999, **360**, 488-499.

165. *Sandostatin LAR International Website For Healthcare Professionals Outside the U.S*, http://www.sandostatin.com/about-sandostatin-lar/what-is-sandostatin-lar.jsp, Accessed 3rd March, 2014.

166. J. W. Wang, Y. Li, Z. G. Mao, B. Hu, X. B. Jiang, B. B. Song, X. Wang, Y. H. Zhu and H. J. Wang, *Patient Prefer. Adherence*, 2014, **8**, 43-51.

167. J. C. Reubi and A. M. Landolt, *J. Clin. Endocrinol. Metab.*, 1989, **68**, 844-850.

168. K. Oberg, L. Kvols, M. Caplin, G. Delle Fave, W. de Herder, G. Rindi, P. Ruszniewski, E. A. Woltering and B. Wiedenmann, *Ann. Oncol.*, 2004, **15**, 966-973.

169. A.-K. Imhof, L. Glück, M. Gajda, A. Lupp, R. Bräuer, H.-G. Schaible and S. Schulz, *Arthritis Rheum.*, 2011, **63**, 2352-2362.

170. F. Barbieri, A. Pattarozzi, M. Gatti, C. Porcile, A. Bajetto, A. Ferrari, M. D. Culler and T. Florio, *Endocrinology*, 2008, **149**, 4736-4746.

171. M. Pfeiffer, T. Koch, H. Schroder, M. Klutzny, S. Kirscht, H. J. Kreienkamp, V. Hollt and S. Schulz, *J. Biol. Chem.*, 2001, **276**, 14027-14036.

172. G. Keri, I. Mezo, A. Horvath, Z. Vadasz, A. Balogh, M. Idei, T. Vantus, I. Teplan, M. Mak, J. Horvath, K. Pal and O. Csuka, *Biochem. Biophys. Res. Commun.*, 1993, **191**, 681-687.

173. G. Kéri, J. Erchegyi, A. Horváth, I. Mezõ, M. Idei, T. Vántus, A. Balogh, Z. Vadász, G. Bökönyi, J. Seprõdi, I. Teplán, O. Csuka, M. Tejeda, D. Gaál, Z. Szegedi, B. Szende, C. Roze, H. Kalthoff and A. Ullrich, *Proc. Natl. Acad. Sci.*, 1996, **93**, 12513-12518.

174. H. Zheng, A. Bailey, M.-H. Jiang, K. Honda, H. Y. Chen, M. E. Trumbauer, L. H. T. Van der Ploeg, J. M. Schaeffer, G. Leng and R. G. Smith, *Mol. Endocrinol.*, 1997, **11**, 1709-1717.

175. D. Cervia, D. Langenegger, E. Schuepbach, M. Cammalleri, P. Schoeffter, H. A. Schmid, P. Bagnoli and D. Hoyer, *Neuropharmacology*, 2005, **48**, 881-893.

176. H. A. Schmid, *Mol. Cell. Endocrinol.*, 2008, **286**, 69-74.

177. R. Cescato, K. A. Loesch, B. Waser, H. R. Mäcke, J. E. Rivier, J. C. Reubi and A. Schonbrunn, *Mol. Endocrinol.*, 2010, **24**, 240-249.

178. G. Weckbecker, I. Lewis, R. Albert, H. A. Schmid, D. Hoyer and C. Bruns, *Nat. Rev. Drug. Discov.*, 2003, **2**, 999-1017.

179. C. Casnici, D. Lattuada, C. Perego, P. Franco and O. Marelli, *Int. J. Immunopharmacol.*, 1997, **19**, 721-727.

180. M. C. Sirianni, B. Annibale, S. Fais and G. Delle Fave, *Peptides*, 1994, **15**, 1033-1036.

181. P. M. van Hagen, E. P. Krenning, D. J. Kwekkeboom, J. C. Reubi, P. J. Anker-Lugtenburg, B. Lowenberg and S. W. Lamberts, *Eur. J. Clin. Invest.*, 1994, **24**, 91-99.

182. D. Ferone, P. M. van Hagen, P. M. van Koetsveld, J. Zuijderwijk, D. M. Mooy, E. G. Lichtenauer-Kaligis, A. Colao, A. J. Bogers, G. Lombardi, S. W. Lamberts and L. J. Hofland, *Endocrinology*, 1999, **140**, 373-380.

183. G. Peluso, O. Petillo, M. A. B. Melone, G. Mazzarella, M. Ranieri and G. F. Tajana, *Neuropeptides*, 1996, **30**, 443-451.

184. S. J. Bhathena, J. Louie, G. P. Schechter, R. S. Redman, L. Wahl and L. Recant, *Diabetes*, 1981, **30**, 127-131.

185. E. Lichtenauer-Kaligis, P. Van Hagen, S. Lamberts and L. Hofland, *Eur. J. Endocrinol.*, 2000.

186. H. Nakamura, T. Koike, K. Hiruma, T. Sato, H. Tomioka and S. Yoshida, *Immunology*, 1987, **62**, 655.

187. S. Sreedharan, K. T. Kodama, K. E. Peterson and E. J. Goetzl, *J. Biol. Chem.*, 1989, **264**, 949-952.

188. K. Hiruma, T. Koike, H. Nakamura, T. Sumida, T. Maeda, H. Tomioka, S. Yoshida and T. Fujita, *Immunology*, 1990, **71**, 480.

189. M. C. Aguila, W. L. Dees, W. E. Haensly and S. M. McCann, *Proc. Natl. Acad. Sci. U. S. A.*, 1991, **88**, 11485-11489.

190. P. J. Fuller and K. Verity, *J. Immunol.*, 1989, **143**, 1015-1017.

191. J. V. Weinstock and D. Elliott, *Eur. J. Endocrinol.*, 2000, **143 Suppl 1**, S15-19.

192. Z. Helyes, E. Pintér, J. Németh, G. Kéri, M. Thán, G. Oroszi, A. Horváth and J. Szolcsányi, *Br. J. Pharmacol.*, 2001, **134**, 1571-1579.

193. V. D. Fitzpatrick and R. L. Vandlen, *J. Biol. Chem.*, 1994, **269**, 24621-24626.

194. J. Dimech, W. Feniuk and P. P. Humphrey, *Br. J. Pharmacol.*, 1993, **109**, 898-899.

195. K. Kaupmann, C. Bruns, F. Raulf, H. P. Weber, H. Mattes and H. Lubbert, *EMBO J.*, 1995, **14**, 727-735.

196. G. Liapakis, D. Fitzpatrick, C. Hoeger, J. Rivier, R. Vandlen and T. Reisine, *J. Biol. Chem.*, 1996, **271**, 20331-20339.

197. M. A. Simmons, *Mol. Interventions*, 2005, 154-157.

198. R. F. Furchgott and P. Bursztyn, *Ann. N. Y. Acad. Sci.*, 1967, **144**, 882-899.

199. J. D. Urban, W. P. Clarke, M. von Zastrow, D. E. Nichols, B. Kobilka, H. Weinstein, J. A. Javitch, B. L. Roth, A. Christopoulos, P. M. Sexton, K. J. Miller, M. Spedding and R. B. Mailman, *J. Pharmacol. Exp. Ther.*, 2007, **320**, 1-13.

200. V. C. Jordan, *J. Natl. Cancer. Inst.*, 2007, **99**, 350-356.

201. D. M. Perez and S. S. Karnik, *Pharmacol. Rev.*, 2005, **57**, 147-161.

202. S. C. Royall, PhD, University of Warwick, 2012.

203. B. A. Hay, B. M. Cole, F. DiCapua, G. W. Kirk, M. C. Murray, R. A. Nardone, D. J. Pelletier, A. P. Ricketts, A. S. Robertson and T. W. Siegel, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 2731-2734.

204. S. P. Rohrer, E. T. Birzin, R. T. Mosley, S. C. Berk, S. M. Hutchins, D.-M. Shen, Y. Xiong, E. C. Hayes, R. M. Parmar, F. Foor, S. W. Mitra, S. J. Degrado, M. Shu, J. M. Klopp, S.-J. Cai, A. Blake, W. W. S. Chan, A. Pasternak, L. Yang, A. A. Patchett, R. G. Smith, K. T. Chapman and J. M. Schaeffer, *Science*, 1998, **282**, 737-740.

205. L. Yang, L. Guo, A. Pasternak, R. Mosley, S. Rohrer, E. Birzin, F. Foor, K. Cheng, J. Schaeffer and A. A. Patchett, *J. Med. Chem.*, 1998, **41**, 2175-2179.

206. A. Christopoulos, *Nat. Rev. Drug Discov.*, 2002, **1**, 198-210.

207. L. T. May, K. Leach, P. M. Sexton and A. Christopoulos, *Annu. Rev. Pharmacol. Toxicol.*, 2007, **47**, 1-51.

208. C. Valant, P. M. Sexton and A. Christopoulos, *Mol. Interventions*, 2009, **9**, 125-135.

209. A. L. Weber and S. L. Miller, *J. Mol. Evol.*, 1981, **17**, 273-284.

210. M. A. Lipson and E. Sondheimer, *J. Org. Chem.*, 1964, **29**, 2392-2394.

211. K. G. Poduska K., Silaev A., Rudinger J., *Collect. Czech. Chem. Commun.*, 1965, **30**, 2410-2433.

212. N. Izumiya, H. Okazaki, I. Matsumoto and H. Takiguchi, *J. Biochem. (Tokyo, Jpn.)*, 1959, **46**, 1347-1354.

213. S. M. Morris, *J. Nutr.*, 2004, **134**, 2743S-2747S.

214. A. Humm, E. Fritsche, S. Steinbacher and R. Huber, *EMBO J.*, 1997, **16**, 3373-3385.

215. D. Cai, K. Deng, W. Mellado, J. Lee, R. R. Ratan and M. T. Filbin, *Neuron*, 2002, **35**, 711-719.

216. D. Kepka-Lenhart, S. K. Mistry, G. Wu and S. M. Morris, *Am. J. Physiol.*, 2000, **279**, R2237-R2242.

217. H. Li, C. J. Meininger, J. R. Hawker, Jr., T. E. Haynes, D. Kepka-Lenhart, S. K. Mistry, S. M. Morris, Jr. and G. Wu, *American journal of physiology. Endocrinology and metabolism*, 2001, **280**, E75-82.

218. J.-Y. Chang, *Eur. J. Biochem.*, 1985, **151**, 217-224.

219. H. Jakubowski, *Biochemistry*, 1999, **38**, 8088-8093.

220. J. W. Craig, M. A. Cherry and S. F. Brady, *J. Bacteriol.*, 2011, **193**, 5707-5715.

221. Y. Kawai, N. Takasuka, K. Akagawa and S. Naito, *Infect. Immun.*, 1996, **64**, 2101-2105.

222. H. Uchisawa, T. Naraoka and T. Ono, *Biochim. Biophys. Acta, Gen. Subj.*, 2007, **1770**, 790-796.

223. A. Shenoi, N. L and R. Christopher, *Indian Pediatr.*, 2001, **38**, 914-918.

224. G. A. Mitchell, L. C. Brody, J. Looney, G. Steel, M. Suchanek, C. Dowling, V. Der Kaloustian, M. Kaiser-Kupfer and D. Valle, *J. Clin. Invest.*, 1988, **81**, 630-633.

225. P. D. Gatfield, E. Taller, D. M. Wolfe and M. D. Haust, *Pediatr. Res.*, 1975, **9**, 488-497.

226. V. G. Oberholzer and A. Briddon, *Clin. Chim. Acta*, 1978, **87**, 411-415.

227. V. Fell and R. J. Pollitt, *Clin. Chim. Acta*, 1978, **87**, 405-409.

228. J. Martens-Lobenhoffer, A. Becker, H. Freude and S. M. Bode-Boger, *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, 2009, **877**, 2284-2289.

229. N. H. Shah and T. W. Muir, *Chem. Sci.*, 2014, **5**, 446-461.

230. J. P. Gogarten, A. G. Senejani, O. Zhaxybayeva, L. Olendzenski and E. Hilario, *Annu. Rev. Microbiol.*, 2002, **56**, 263-287.

231. M. E. Coates, R. Fuller, G. Harrison, M. Lev and S. Suffolk, *Br. J. Nutr.*, 1963, **17**, 141-150.

232. N. W. Solomons, M. Mazariegos, K. H. Brown and K. Klasing, *Nutr. Rev.*, 1993, **51**, 327-332.

233. J. H. Humphrey, *Lancet*, 2009, **374**, 1032-1035.

234. M. A. Mendall, *BMJ*, 1998, **316**, 953-954.

CHAPTER 2: *C*-TERMINAL LACTAM PEPTIDES

2.1 LINEAR PEPTIDE SYNTHESIS

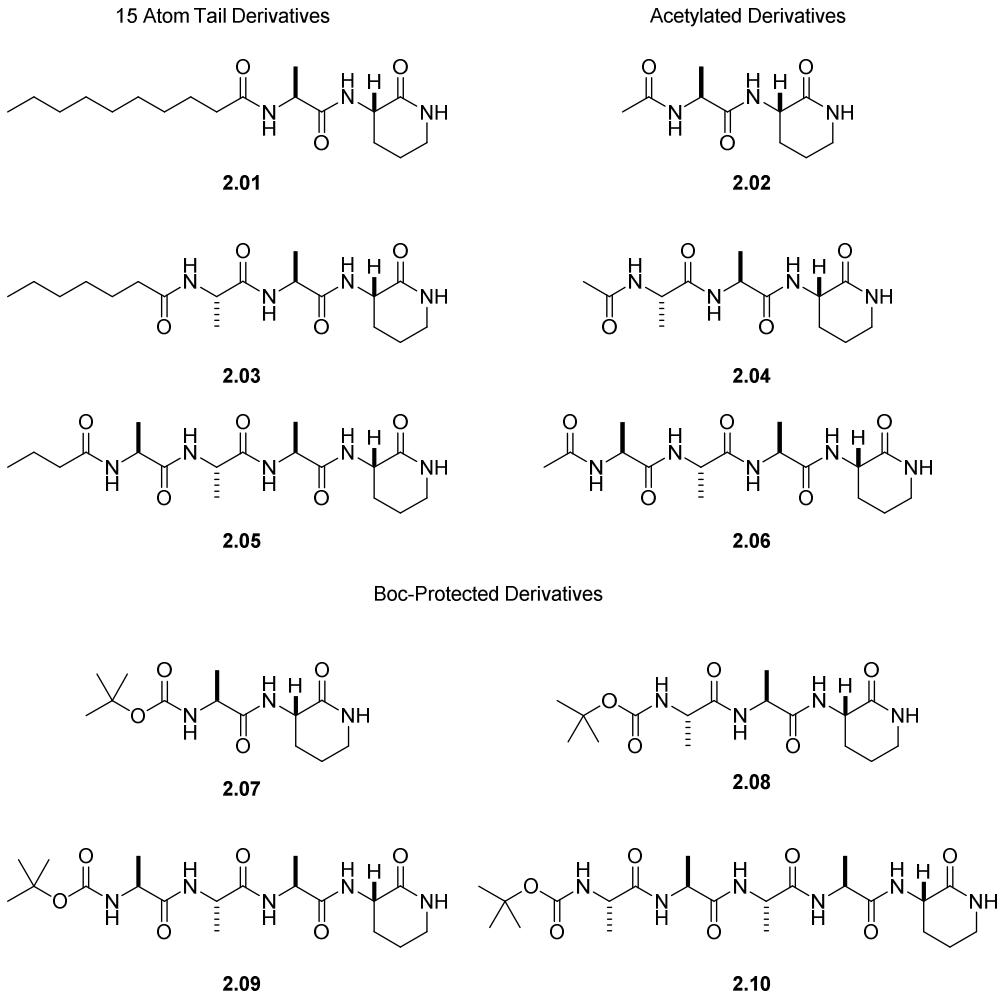
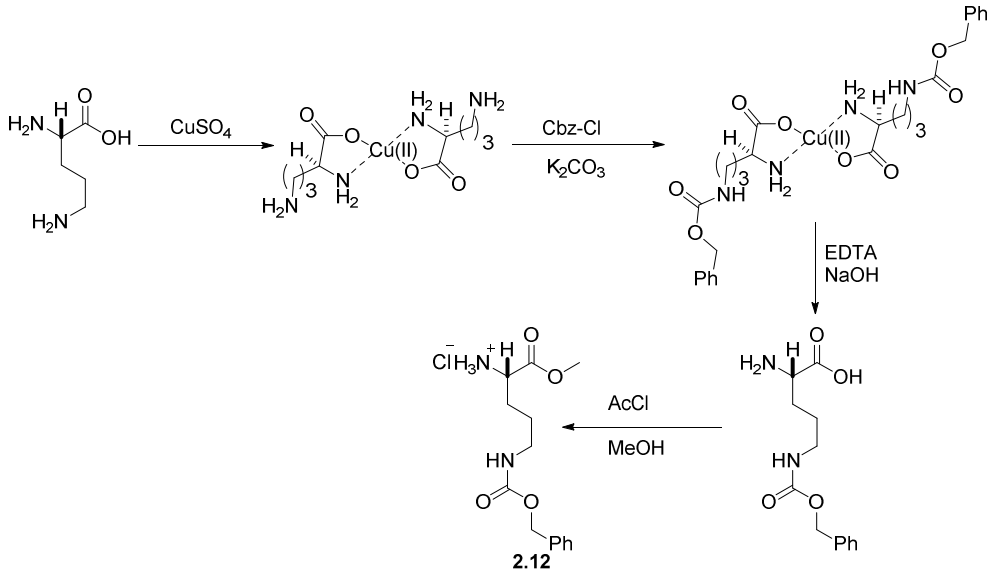


Figure 24: The 10 CTLPs proposed for synthesis

The first nine compounds (**2.01 - 2.09**, Figure 24) were synthesised using *C* to *N* linear solution phase peptide synthesis. L-Alanine was chosen as a simple example and because of the ability of even short peptide stretches to form regular secondary protein structures, with tetraalanine calculated to form α-helices in some solvents, and we hoped to see how this affected $sstr_2$ binding and somatotaxin activity.[1, 2] As the (*S*)-3-amino-2-piperidones are very water soluble, it was decided to use δ-*N*-benzyloxycarbonyl-L-ornithine methyl ester hydrochloride, **2.12**, as a more lipophilic headgroup throughout the synthesis. This was then deprotected and spontaneously ring closed to provide the desired lactam products. The synthesis started with ornithine being selectively protected using a copper (II) chelate, leaving only the δ-nitrogen free to react with the benzyl chloroformate (Scheme 1).[3, 4] The copper was then sequestered using EDTA to afford the δ-*N*-benzyloxycarbonyl-L-ornithine as the acid, which was readily converted to the methyl ester hydrochloride **2.12** using methanolic HCl.



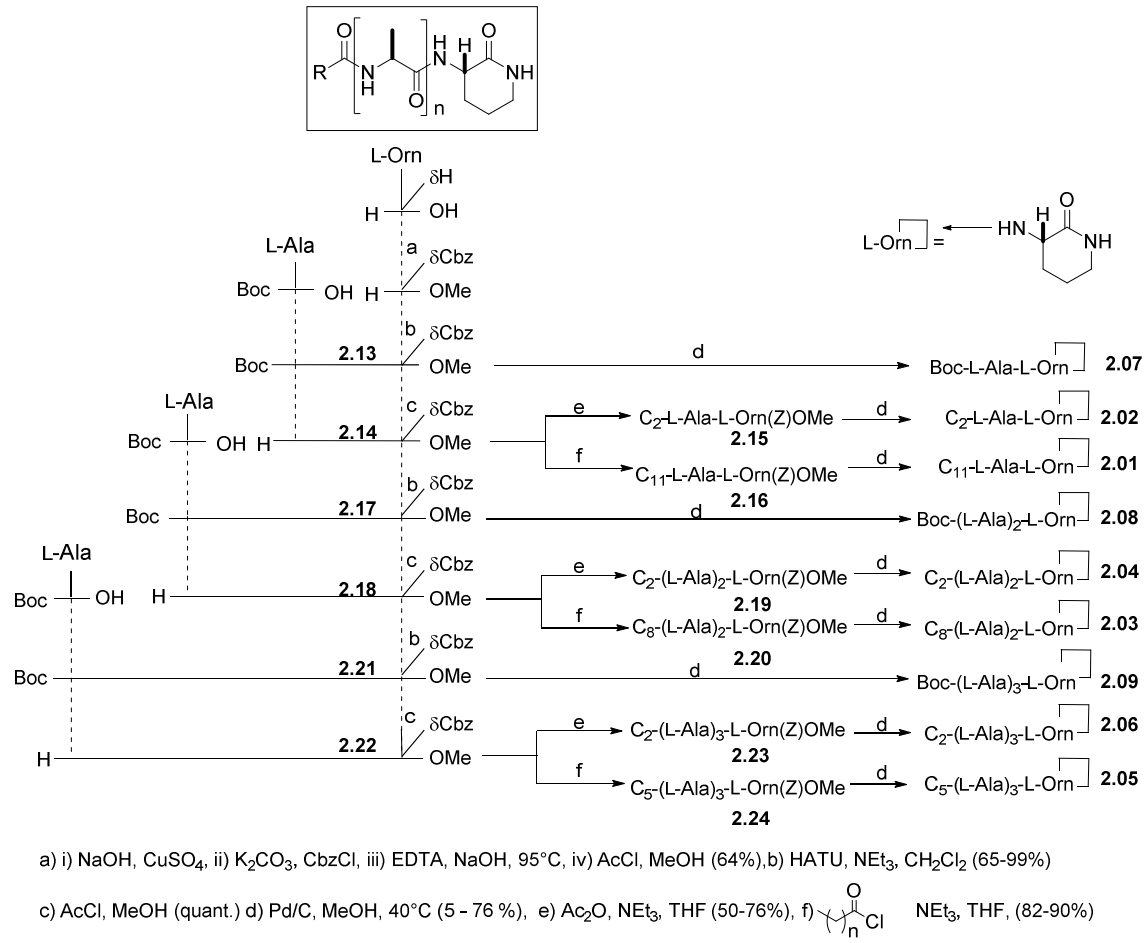
Scheme 1: The route to the selectively mono-protected ornithine 2.12 using a copper (II) chelate

A combination of α-*N*-terminal Boc protection and a *C*-terminal methyl ester were chosen as compatible protecting groups, as *N*-*tert*-butoxy-L-alanine (Boc-L-alanine) is cheap and readily available, and the Boc group could be deblocked easily using methanolic HCl without affecting other functional groups.

The peptide chains were built using successive rounds of peptide coupling using Boc-L-alanine, HATU and triethylamine (Scheme 2). *O*-(7-Aza-benzotriazol-1-yl)-*N*, *N*, *N'*, *N'*-tetramethyluronium hexafluorophosphate (HATU) is one of large number of commercially available peptide coupling agents based on 1-hydroxybenzotriazole (HOBt). It consists of a guanidinium salt of a 1-hydroxy-7-azabenzotriazole (HOAt), and is considered to be superior to HOBt in terms of reduction of racemisation and rate of reaction.[5-8] The carboxylic acid of the first amino-acid reacts rapidly with the guanidinium functionality of HATU to form an activated ester. This is rapidly intercepted by the HOAt moiety to give a second ester with the elimination of tetramethylurea (TMU).[9] The coupling is completed with the nucleophilic substitution by a free amine to give the coupled product.

The peptide couplings performed between Boc-L-alanine and the growing peptide generally proceeded with very high conversions but the TMU by-product initially proved difficult to remove. A literature search showed that it was possible to exploit the comparative solubility of TMU in water and toluene.[10] Concentration of the crude reaction mixture, followed by dissolution of the residue in toluene and several aqueous washes (pH 2 buffer, saturated carbonate solution, water) followed by concentration *in vacuo* and a toluene azeotrope generally gave the coupled product in very high yields and purity, without the need for column chromatography or further drying. Boc-deprotection was achieved easily using methanolic HCl. Each deprotected peptide underwent three further reactions: a reaction with acetic anhydride to give the capped peptide (e.g. **2.15**, Scheme **2**), reaction with the relevant acyl chloride to give a 15 atom tail group (e.g. **2.16**, Scheme **2**) or a further peptide coupling with Boc-l-alanine (e.g. **2.17**, Scheme **2**).

Once the final tail group was added, compounds were hydrogenated over palladium to deprotect the γ-NH_2, leading to spontaneous ring closure and loss of the methyl ester to give the desired series of CTLPs.



Scheme 2: The linear synthesis of the first nine CTLPs

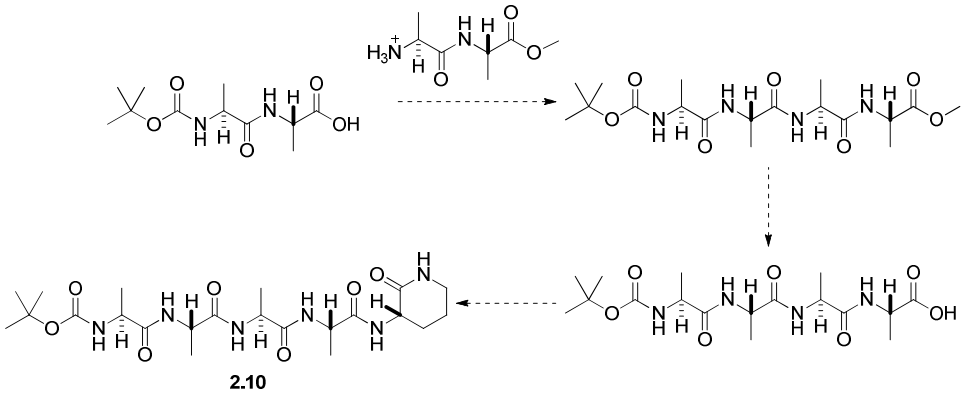
Using this method, the first nine CTLPs were synthesised in sufficient quantities for full characterisation and further testing. However, though the yields for the peptide coupling and acylation reactions were generally good, the overall yields were often poor. In addition, with the exception of the four simplest dipeptides, all needed purification by reverse phase HPLC in order to remove residual inorganics and impurities from the coupling steps and final hydrogenation. With the addition of longer tailgroups and more amino-acids the compounds became less easy to manipulate and purify. The tri-alanine tetrapeptides (**2.22 - 2.24**) were much less soluble than the di- and tripeptides, to the extent that they were insoluble in the toluene used in the removal of TMU and had to be stirred vigorously as a suspension in toluene and water at high temperatures and filtered to remove both the TMU and

the triethylamine hydrochloride salt. The tetra-alanine pentapeptide was so insoluble as to form a gelatinous solid in both DCM and MeOH, preventing complete reaction.

In addition, even the relatively soluble small compounds were unexpectedly difficult to hydrogenate at the final step, requiring at least 2 days under a constant pressure of hydrogen rather than hours observed in comparable reactions within the research group. It seems likely that the peptides were complexing to the catalyst, preventing complete reaction of the substrate and leading to reduced yields (5 - 76%).

2.2 BLOCK SYNTHESIS OF PENTAPEPTIDES USING ETHYL HYDROXIMINO-2-CYANOACETATE

The linear peptide coupling method used to produce the first 9 CTLPs suffered from a variety of disadvantages, and it was obvious that in order to obtain the 6-membered lactam with a tetrapeptide tail, a different route will have to be attempted. Initially, we planned to build up the tetra-alanyl chain using convergent block synthesis of a pair of dipeptides (Scheme 3).



2.10

Scheme 3: Proposed synthesis of the tetraalanyl CTLP, 2.10

As the final hydrogenation and ring closure step was problematic, it was hoped to bypass this by performing a coupling directly between the lactam headgroup and the tetrapeptide chain. Finally, a different peptide coupling reagent was sought, as the tetramethyl urea (TMU) by-product from HATU became increasingly difficult to remove as the peptide chain length increased.

A peptide coupling method proposed by Pu *et al.* provided a viable route to the pentapeptide.[11] The reaction was performed in aqueous EtOH, which led to greatly improved solubility of both the intermediates and the products. EDCI was used as the coupling agent, with the advantage of producing readily water-soluble by-products. Rather than using the benzotriazole HOBt as a racemisation suppression agent, it was decided to use ethyl hydroximino-2-cyanoacetate (Oxyma, **Figure 25**).[12]

This coupling agent works in a similar way to the benzotriazole based HOBt and HOAt coupling agents with improved suppression of epimerization, but does not have the same risk of explosion, and hence is cheaper and easier to obtain.[13] The reported decrease in racemization in reactions using Oxyma is likely to be due to the nucleophilic, yet stable, oxime anion, reducing the rate of 5-(4*H*)-oxazolone formation (Figure 25).[14]

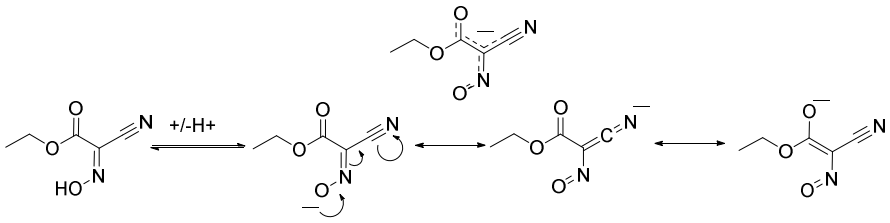
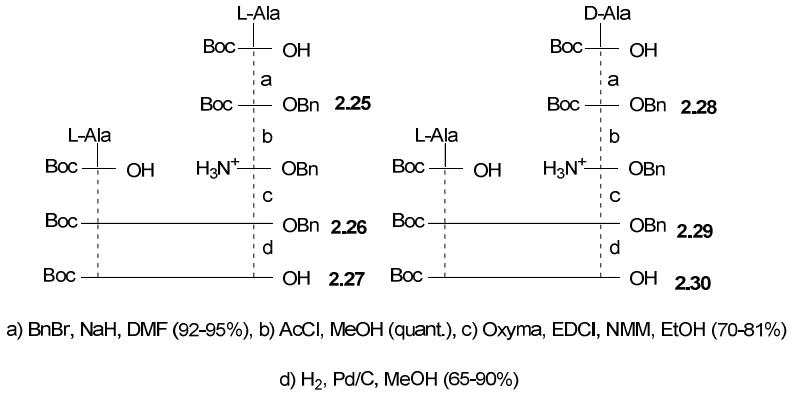


Figure 25: Resonance forms of deprotonated Oxyma[14]

However, unlike linear *C* to *N* peptide synthesis, where epimerisation is largely avoided by using urethane protected amino-acids (**Scheme 2**), block peptide couplings risk epimerisation of the *C*-terminal amino-acid during the coupling reactions.

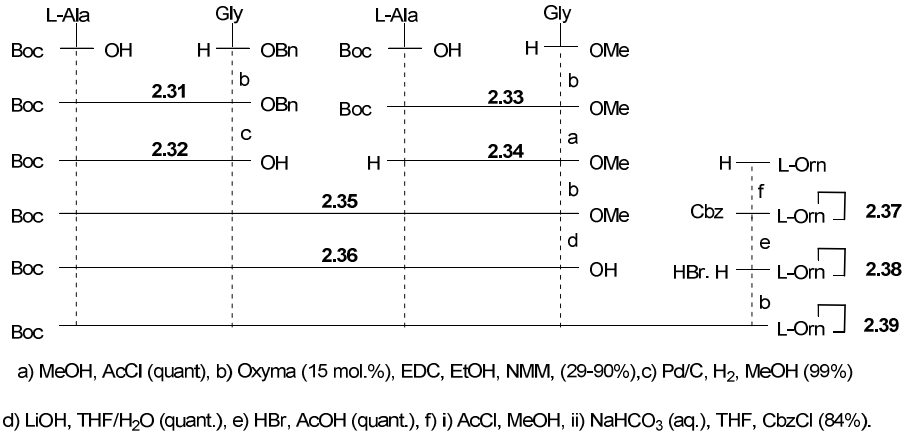
As a starting point to test racemisation with this method, we synthesised both *N-tert*-butoxy-L-alanyl-L-alanine, **2.27**, and *N-tert*-butoxy-L-alanyl-D-alanine, **2.30** (Scheme 4).



Scheme 4: The synthesis of two diastereomers of Boc-L-alanylalanine

Unfortunately, there were no discernible differences between these two diastereomers by ^{1}H NMR spectroscopy.[15] At this point it was noted that the harsh conditions used for the formation of the benzyl ester **2.25** appeared to have resulted in significant racemization (based on optical rotation, $[\alpha]_D^{28}$ -6.1 (c = 1.9, MeOH) (lit.[16] $[\alpha]_D^{25}$ -40.5 (c = 2.0, MeOH)). However, the ^{1}H NMR spectrum of the resulting dipeptide diastereomer **2.27** showed no obvious additional peaks for the *N-tert*-butoxy-D-alanyl-L-alanine diastereomer. The ^{13}C NMR spectrum showed the appearance of additional peaks, but these were in no way distinctive enough to be a reliable method for assessing epimerization.

As these observations meant we would be unable to easily assess what level of epimerisation was occurring at the *C*-terminal amino-acids, we sidestepped the issue by replacing alternate alanine residues with glycine residues (Scheme 5). As glycine does not have a stereogenic centre, it could be used at the *C*-terminal end of the peptide without epimerisation being a concern. It was also hoped that the mix of amino-acids in the peptide chain would improve the solubility of the final peptides, but still retain a 'native peptide-like' character.



Scheme 5: Block synthetic route to the pentapeptide CTLP, **2.39**

Initially, the *N*- and *C*- protected alanylglycine dipeptide was produced as both the benzyl and methyl esters using this method (**2.31** and **2.33**, Scheme 5). It became apparent that, despite only catalytic amounts of Oxyma being used, by-products were formed during the dipeptide formation that were difficult to completely remove during work up. This has been previously commented on, but we hoped that the side reactions leading to these impurities would be suppressed if Oxyma was present in substoichiometric amounts.[13, 17] However, we found that both dipeptides required further purification by silica chromatography to remove these persistent by-products. Boc-deprotection of the methyl ester dipeptide was performed with methanolic HCl, and the benzyl ester of the *N*-terminal dipeptide removed using catalytic hydrogenation. Coupling was again performed using the catalytic Oxyma/EDCI method to give the tetrapeptide methyl ester (**2.35**, Scheme 5). The low yield at this step is possibly due to the formation of diketopiperazines, a known side products of couplings involving glycine-containing dipeptides.[18, 19]

While the *C*-terminal benzyl ester would potentially have been easier to isolate and purify due to increased organic solubility compared to the methyl ester, it was decided to avoid the use of palladium catalysed hydrogenation to produce the *C*-deprotected tetrapeptide after the difficulties experienced in the previous syntheses. To avoid loss of the tetrapeptide during aqueous work-up, the crude reaction mixture

was purified by column chromatography in reasonable yield. Hydrolysis was achieved using aqueous lithium hydroxide in EtOH and, as isolation of the product proved problematic, the crude carboxylic acid was used in solution without further purification. After a number of attempts, the crude pentapeptide was synthesised by a final coupling using an excess of the lactam. This required purification by column chromatography and reverse phase HPLC to give the final tetrapeptide, **2.39**, in 1 % overall yield over 5 steps (Scheme 5).

2.3 CONCLUSIONS

In all, ten CTLPs were synthesised. Ultimately, due to a change in direction by our industrial collaborators, it has not yet been possible to assess the $sstr_2$ binding or somatotaxin activity of these synthesised compounds. However, in the process of synthesising these peptides, we identified a number of issues that warranted further investigation.

A major concern was maintaining the chiral integrity of the stereocentre of the lactam and the amino-acid chain. The linear *C* to *N* terminal synthesis would be expected to have minimal levels of epimerisation, as the *C*-terminal amino-acids were supplied as the urethane-protected monomers, which are much less prone to epimerisation than other amides.[20] This linear method suffered from extremely low overall yields and issues with purification, and the convergent block-synthesis of the peptides still appeared to provide a more useful strategy for the production of CTLPs. Unfortunately, our inability to accurately assess levels of epimerisation limited us to the use of glycine as the *C*-terminal amino-acid.

A recent review listed over 280 different peptide coupling agents, used both in solution and solid phase.[21] Despite a huge quantity of research, epimerisation, side-reactions and removal of by-products remain a problem. Even with advances in solid phase peptide synthesis, the process is still wasteful and size of peptide limited. Amide bond formation is still seen as expensive and clumsy, and a recent review stated ‘amide bond formation avoiding poor atom economy reagents’ as a

fundamental challenge for organic chemistry.[22, 23] As implied by the huge diversity of coupling agents available, none of the current reagents provide a particular advantage for a general method of peptide bond formation.[24] Those related to HOBt (the so-called uronium/aminium salts), and the related phosphonium and ammonium salts all rely on the *in situ* formation of a *C*-terminal active ester, and hence are likely to induce a certain level of epimerization, no matter how low. Obviously, all synthetic methods for amide bond preparation require some activation of the acid, and hence run some risk of epimerisation, but we felt that moving away from the 'onium' based reagents could provide a more robust alternative.

While investigating new methods of performing the final amide bond formation between the lactam and the amino-acid tailgroup, we additionally hoped to quantify the level of epimerisation occurring during the peptide coupling, and so minimise it as far as possible.

2.4 REFERENCES

1. H. Stymme, G. Wettermark, R. Schor and C. W. David, *J. Phys. Chem.*, 1973, **77**, 3033-3035.
2. N. C. Pace and M. J. Scholtz, *Biophys. J.*, 1998, **75**, 422-427.
3. A. Rosowsky and J. E. Wright, *J. Org. Chem.*, 1983, **48**, 1539-1541.
4. A. Rosowsky, C. M. Vaidya, H. Bader, J. E. Wright and B. A. Teicher, *J. Med. Chem.*, 1997, **40**, 286-299.
5. L. A. Carpino, A. El-Faham and F. Albericio, *Tetrahedron Lett.*, 1994, **35**, 2279-2282.
6. Y. M. Angell, C. García-Echeverría and D. H. Rich, *Tetrahedron Lett.*, 1994, **35**, 5981-5984.
7. L. A. Carpino, *J. Am. Chem. Soc.*, 1993, **115**, 4397-4398.
8. L. A. Carpino, A. El-Faham, C. A. Minor and F. Albericio, *J. Chem. Soc., Chem. Commun.*, 1994, 201-203.

9. P. Li and J. C. Xu, *J. Pept. Res.*, 2001, **58**, 129-139.

10. N. K. Yee, V. Farina, I. N. Houpis, N. Haddad, R. P. Frutos, F. Gallou, X.-j. Wang, X. Wei, R. D. Simpson, X. Feng, V. Fuchs, Y. Xu, J. Tan, L. Zhang, J. Xu, L. L. Smith-Keenan, J. Vitous, M. D. Ridges, E. M. Spinelli, M. Johnson, K. Donsbach, T. Nicola, M. Brenner, E. Winter, P. Kreye and W. Samstag, *J. Org. Chem.*, 2006, **71**, 7133-7145.

11. Y. J. Pu, R. K. Vaid, S. K. Boini, R. W. Towsley, C. W. Doecke and D. Mitchell, *Org. Process Res. Dev.*, 2008, **13**, 310-314.

12. M. Itoh, *Bull. Chem. Soc. Jpn.*, 1973, **46**, 2219-2221.

13. R. Subirós-Funosas, R. Prohens, R. Barbas, A. El-Faham and F. Albericio, *Chem. - Eur. J.*, 2009, **15**, 9394-9403.

14. S. N. Khattab and M. F. Fathalla, *J. Chem. Soc. Pak.*, 2011, **33**, 324-332.

15. B. Weinstein and A. E. Pritchard, *J. Chem. Soc., Perkin Trans. 1*, 1972, 1015-1020.

16. S.-T. Chen and K. T. Wang, *J. Chem. Soc., Chem. Commun.*, 1988, 327-328.

17. R. Subiros-Funosas, G. A. Acosta, A. El-Faham and F. Albericio, *Tetrahedron Lett.*, 2009, **50**, 6200-6202.

18. E. Gross and J. Meienhofer, *The Peptides: Major Methods of Peptide Bond Formation*, Academic Press, 1979.

19. S.-Y. Han and Y.-A. Kim, *Tetrahedron*, 2004, **60**, 2447-2467.

20. F. L. Scott, R. E. Glick and S. Winstein, *Experientia*, 1957, **13**, 183-185.

21. A. El-Faham and F. Albericio, *Chem. Rev. (Washington, DC, U. S.)*, 2011, **111**, 6557-6602.

22. V. R. Pattabiraman and J. W. Bode, *Nature*, 2011, **480**, 471-479.

23. D. J. C. Constable, P. J. Dunn, J. D. Hayler, G. R. Humphrey, J. J. L. Leazer, R. J. Linderman, K. Lorenz, J. Manley, B. A. Pearlman, A. Wells, A. Zaks and T. Y. Zhang, *Green Chem.*, 2007, **9**, 411-420.

24. J. Hachmann and M. Lebl, *Pept. Sci.*, 2006, **84**, 340-347.

2.5 EXPERIMENTAL

All the reagents and solvents used were purchased from the Sigma-Aldrich, Alfa-Aesar, TCI, Bachem or Fluorochem Chemical Company and were used as received unless stated otherwise. pH 2 buffer was made as a solution of 0.75 M of Na_2SO_4 and 0.25 M H_2SO_4 in H_2O.

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AVII-700 MHz, AVIII-600 MHz, DRX-500 MHz, DPX-400 MHz or DPX-300 MHz Fourier transform spectrometer at room temperature unless stated otherwise. Chemical shifts are quoted in parts per million (ppm) downfield from tetramethylsilane. Solvents were used as an internal standard when assigning NMR spectra (δ_H: $CDCl_3$ 7.26 ppm, CD_3OD 3.31 ppm, DMSO-d_6 2.50 ppm, D_2O 4.79 ppm, d_1-TFA 11.5 ppm; δ_C: $CDCl_3$ 77.1 ppm, CD_3OD 49.0 ppm, DMSO-d_6 39.5 ppm, d_1-TFA 164.2, 116.6 ppm). Coupling constants (*J*) are quoted in Hertz (Hz), rounded to the nearest 0.5 Hz. Abbreviations used in the descriptions of spectra are as follows; s = singlet, d = doublet, t = triplet, q = quartet, quin. = quintet, sept. = septet, oct. = octet, m = multiplet, br = broad, i = ipso, o = ortho, m = meta, p = para, ax. = axial and eq. = equatorial. ^{13} C-NMR spectra were recorded with broadband proton decoupling and spectra were assigned on the basis of COSY, PENDANT, HMQC and HMBC spectra. In aromatic characterisations, the ipso carbon is taken to be the carbon bonded to the group with the highest molecular weight

Infrared spectra were recorded on a Nicolet Avatar 320 FT-IR spectrophotometer using EZ OMNIC software package 1, Bruker ALPHA Platinum ATR spectrophotometer or Perkin ELMER Spectrum 100 FT-IR spectrophotometer using OPUS software and are quoted in wavenumber (cm^{-1}).

Optical rotations were recorded on an Optical Activity Ltd. AA-1000 millidegree auto-ranging polarimeter (using the sodium D line; 589 nm) and $[\alpha]_D$s are given in

units of 10^{-1}deg cm^2 g^{-1}. The samples were made using spectroscopic grade MeOH, $CHCl_3$ or H_2O.

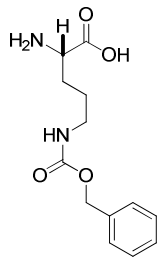
ESI mass spectra were obtained on a Bruker Esquire 2000 mass spectrometer or an Agilent 6130B single Quad (ESI)**.** HRMS ESI spectra were obtained by Dr Lijiang Song, Mr Philip Aston or Dr Rebecca Wills using a Bruker micro-TOF ESI attached to a time of flight (TOF) analyser.

HPLC data was obtained on a Varian Prostar 335LC detector using an ChiralPak AS column, (4.6mm x 250mm) using a solvent system of 1: 1 (n-hexane: iso-propyl alcohol) and a flow rate of 0.5mL / minute. Reverse Phase HPLC was performed on an Agilent 1100, with an Agilent Zorbax RP-C18 Column, 100x21mm, 5um, UV at 220nm.Flow rate: 5ml/min, Solvent: water(A) and ACN (B) both with 0.1% FA, Gradient (B%): 0-10mins, 20% B; 10-12mins, 20-80%B; 12-17mins, 80%B; 17-20mins, 80 to 20% B, then equilibrate for 15min before next run**.**

Melting points for solid crystalline products were determined on a Stuart Scientific SMP10 Digital Melting Point Apparatus, with three runs of each compound, and a range given in ˚C rounded to the nearest degree. They are uncorrected.

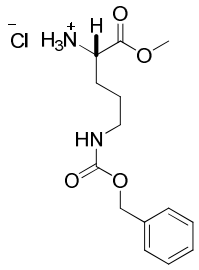
Thin Layer Chromatography (TLC) was performed using silica (0.25 mm) coated aluminium plates.

δ-*N*-(Benzyloxycarbonyl)-L-ornithine 2.11



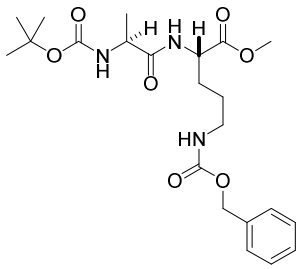
Prepared according to the method of Rosowsky *et al.*[1] Aqueous NaOH solution (0.5M, 200 mL, 100 mmol) was added to L-ornithine hydrochloride (16.9 g, 100 mmol) with stirring to give a clear, colourless solution. $CuSO_4.H_2O$ (12.5 g, 50.0 mmol) was added and the reaction mixture was stirred at room temperature for 40 minutes. K_2CO_3 (13.8 g, 100 mmol) was added, followed by benzyl chloroformate (19.0 mL, 128 mmol) and the mixture stirred at room temperature overnight. The resulting purple precipitate was isolated by filtration and washed with MeOH (2 x 50 mL). The filtercake was added to a solution of EDTA (14.6 g, 50.0 mmol) in aqueous sodium hydroxide solution (0.25M, 400 mL, 100 mmol) to give a purple slurry. After heating to 95 °C for 1 hour, the reaction mixture was cooled to room temperature. The mixture was filtered and the crude product isolated by filtration as a sticky blue solid. This was washed with H_2O (10 x 300 mL) until no further blue colour was observed to give the free amine **2.11** as a white solid (17.1 g; 63.9 mmol, 64 %); m.p. 257 - 258 ºC (lit.[2] 255 - 256 °C); υ_{max}/cm^{-1}(neat) 3328 (N-H), 2945 (broad O-H), 1683 (acid C=O), 1606 (amide C=O), 1535 (N-H); δ_H (400 MHz, d_1-TFA) 7.60 - 6.90 (5H, m, 5 x Ar C*H*), 5.28 (2H, s, CH_2Ph), 4.46 (1H, t, J 6.5 Hz, $CHNH_2$), 3.44 (2H, t, J 6.5 Hz, CH_2NH), 2.51 - 2.11 (2H, m, $CHCH_2CH_2$), 2.06 - 1.77 (2H, m, $CHCH_2CH_2$); δ_C (100 MHz, d_1-TFA) 173.0 (CO_2H), 160.2 (CO_2Bn), 134.5 (ipso *C*), 128.6, 128.3, 127.7 (*ortho, meta* and *para C*H), 69.1 (CH_2Ph), 53.8 ($CHNH_2$), 39.8 (CH_2NH), 24.4, 22.1, (2 x CH_2,); m/z (ESI+) 289.1 ([M+Na]+, 100.0%), 267.1 ([M+H]+, 15.2%); HR-ESIMS: calculated for $C_{11}H_{19}N_2O_4$: 267.1339, found: 267.1346 $[M+H]^+$. Data are consistent with that previously reported. [2, 3]

δ-*N*-(Benzyloxycarbonyl)-L-ornithine methyl ester hydrochloride 2.12



Acetyl chloride (35 mL, 492.3 mmol) was added dropwise with stirring and cooling in an icebath to MeOH (450 mL). δ-*N*-(Benzoyloxycarbonyl)-L-ornithine **2.11** (17.1 g, 63.9 mmol) was added to this solution and stirred at room temperature overnight. The reaction was concentrated *in vacuo* to afford the **2.12** as a white fluffy solid (20.2 g, 63.8 mmol, quantitative yield); m.p. 137 - 138ºC, (lit.[1] 138 - 139 °C); $[\alpha]_D^{34}$ +16.0 (c = 0.59, MeOH), (lit.[4] $[\alpha]_D^{20}$ +13.8 (c = 2.0, MeOH)); υ_{max}/cm^{-1} (neat) 3336 (amide N-H), 3016 (N^+-H), 1742 (ester C=O), 1689 (amide C=O), 1535 (N-H); δ_H (400 MHz, DMSO-d_6) 8.59 (3H, br. s., N*H*$_3$), 7.48 - 7.20 (6H, m, 5 x Ar C*H*, CH_2N*H*), 5.01 (2H, s, C*H*$_2$Ph), 4.00 (1, t, *J* 6.5 Hz, C*H*CH_2), 3.73 (3H, s, OC*H*$_3$), 3.00 (2H, q, *J* 6.5 Hz, C*H*$_2$NH), 1.86 - 1.70 (2H, m, C*H*$_2$$CH_2$NH), 1.62 - 1.36 (2H, m, CHC*H*$_2$); δ_C (100 MHz, DMSO-d_6) 169.8 (CO_2Me), 156.1 (*C*O_2Bn), 137.2 (*ipso C*), 128.3, 127.7, 127.7 (*ortho*, *meta* and *para C*H), 65.2 (*C*H_2Ph), 52.7 (O*C*H_3), 51.6 (*C*HCH_2), 40.1 (*C*H_2NH), 27.4 (*C*$H_2$$CH_2$NH), 24.9, (CH*C*$H_2$); m/z (ESI+) 281.1 ([M-Cl]+, 100%); HR-ESIMS: calculated for $C_{14}H_{21}N_2O_4$: 281.1496, found: 281.1493 $[M-Cl]^+$. Data are consistent with that previously reported.[1]

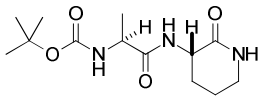
α-*N*-(*N*-*tert*-Butoxycarbonyl-L-alanyl)-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester 2.13



N-*tert*-Butoxycarbonyl-L-alanine (5.97 g, 31.6 mmol) and HATU (12.0 g, 31.6 mmol) were stirred in CH_2Cl_2 (90 mL). After 5 minutes δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester hydrochloride **2.12** (10.0 g, 31.6 mmol) was added and the suspension cooled to 0 °C using an ice-salt bath. The mixture was stirred at 0 °C for 10 minutes and triethylamine (9.60 mL, 94.8 mmol) was added slowly. The yellow suspension was stirred at 0 °C to room temperature until the reaction mixture became a clear yellow solution (1-3 hours) indicating completion. pH 2 buffer (50 mL) was added and the phases separated. The organic layer was washed with pH 2 buffer (50 mL), aqueous saturated sodium $NaHCO_3$ solution (2 x 50 mL) and water (50 mL). The organic phase was concentrated *in vacuo* and the yellow residue taken into toluene (100 mL). pH 2 buffer (50 mL) was added and the toluene layer was washed as before. The organic phase was concentrated *in vacuo* and dried by toluene azeotrope (4 x 50 mL). Residual solvent was removed under vacuum to afford **2.13** as a colourless oil that crystallised on standing to give a colourless crystalline solid (12.6 g, 27.9 mmol, 88 %); m.p. 77 - 79 ºC; $[\alpha]_D^{26}$ -3.8 (c = 1.0, $CHCl_3$), (lit.[5] $[\alpha]_D^{20}$ -5.0 (c = 1.74, $CHCl_3$)) ; υ_{max}/cm^{-1} (neat) 3325 (N-H), 1736 (ester C=O), 1689 (carbamate C=O), 1652 (amide C=O), 1519 (N-H); δ_H (400 MHz, $CDCl_3$) 7.19 - 7.05 (5H, m, 5 x Ar C*H*), 6.90 (1H, br. s., CH_2CHNH), 5.17 (2H, br.s., CH_3CHNH, CH_2NH), 5.02 (2H, s, CH_2Ph), 4.50 (1H, td, *J* 8.0, 5.5 Hz, CH_2CHNH), 4.18 - 4.07 (1H, m, $CHCH_3$), 3.65 (3H, s, OCH_3), 3.12 (2H, q, *J* 6.5 Hz, CH_2NH), 1.88 - 1.76 (1H, m, CH_2CH) 1.69 - 1.55 (1H, m, CH_2CH), 1.54 - 1.41 (2H, CH_2CH_2NH), 1.36 (9H, s, $C(CH_3)_3$), 1.27 (3H, d, *J* 7.0 Hz, $CHCH_3$); δ_C (100 MHz, $CDCl_3$) 172.8, 172.5

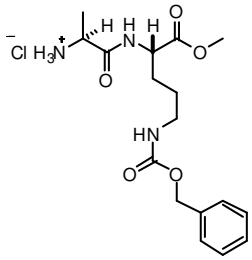
(CH*C*ONH, *C*O_2Me), 156.6, 155.6 (*C*O_2Bn, *C*$O_2{}^t$Bu), 136.7 (*ipso C*), 128.5, 128.1 (*ortho*, *meta* and *para C*H), 80.1 (*C*$(CH_3)_3$), 66.6 (Ph*C*H_2), 52.4 (O*C*H_3), 51.8 (NH*C*HCH_2), 50.1 (NH*C*HCH_3), 40.4 (NHCH_2), 29.4 (*C*H_2), 28.3 (C(*C*$H_3)_3$), 25.9 (*C*H_2), 18.2 (NHCH*C*H_3); m/z (ESI+) 474.1 ([M+Na]+, 100.0%), 374.1 ([M-Ph]+, 21.0%); HR-ESIMS: calculated for $C_{22}H_{33}N_3O_7Na$: 474.2214, found: 474.2211 $[M+Na]^+$. This compound has been previously reported,[5] but without any spectroscopic data.

(*S*)-3-[(*N-tert*-butoxycarbonyl)-L-alanyl]-aminopiperidine-2-one 2.07



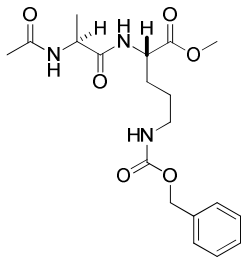
α-*N*-(*N-tert*-Butoxycarbonyl-L-alanyl)-δ-*N-(*benzyloxycarbonyl)-L-ornithine methyl ester **2.13** (1.5 g, 3.32 mmol) was dissolved in MeOH (20 mL) and catalytic palladium (~20 mg, 10% on activated carbon) was added. The reaction vessel was purged with H_2 and the reaction mixture was stirred under 1 atm H_2 atmosphere for 72 hours. The reaction was filtered through celite and the filtrate concentrated *in vacuo* to give sticky white crystals. This was purified by silica chromatography (5% MeOH:$CHCl_3$) to give **2.07** as a colourless crystalline solid (0.31 g, 1.05 mmol, 32 %); m.p. 136 - 137°C; $[\alpha]_D^{26}$ +30.3 (c = 0.31, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3285 (N-H), 1664, (amide C=O), 1511 (N-H); δ_H (400 MHz, $CDCl_3$) 7.63 (1H, d, *J* 5.0 Hz, N*H*CHCH_2), 7.17 (1H, br. s., N*H*CH_2), 5.70 (1H, d, *J* 6.5 Hz, N*H*CHCH_3), 4.23 - 4.19 (2H, m, C*H*CH_3, NHC*H*CH_2), 3.23 (2H, t, *J* 5.0 Hz, NHC*H*$_2$), 2.34 - 2.24 (1H, m, NHCHC*H*$_2$), 1.88 - 1.73 (2H, m, NHCH_2C*H*$_2$), 1.55 (1H, qd, *J* 11.5, 5.5 Hz, NHCHC*H*$_2$), 1.35 (9H, s, C(C*H*$_3)_3$), 1.27 (3H, d, *J* 7.0 Hz, CHC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 173.9 (*C*ONH), 171.7 (*C*ONH), 155.4 (*C*$O_2{}^t$Bu), 79.8 (*C*$(CH_3)_3$), 50.1, 50.0 (NH*C*HCH_3, NH*C*HCH_2), 41.5 (NH*C*H_2), 28.3 (C(*C*$H_3)_3$), 27.1, 20.9 (2 x *C*H_2), 18.8 (CH*C*H_3); m/z (ESI+) 474.1 ([M+Na]+, 100.0%), 374.1 ([M-Ph]+, 21.0%); m/z (ESI+) 307.9 ([M+Na]+, 100.0%); HR-ESIMS: calculated for $C_{13}H_{23}N_3O_4Na$: 308.1581, found: 308.1581 $[M+Na]^+$.

α-*N*-(L-Alanyl)-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester hydrochloride 2.14



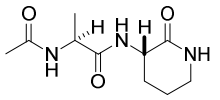
α-*N*-(*N*-*tert*-Butoxycarbonyl-L-alanyl)-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester **2.13** (12.6 g, 27.9 mmol) was deprotected by stirring in methanolic HCl (2M, 60.0 mL) for 3 hours at room temperature. Concentration *in vacuo* followed by trituration in Et_2O gave the amine **2.14** as a white semi-crystalline oil (10.3 g; 25.9 mmol, 93 %); $[\alpha]_D^{30}$ +6.6 (c = 1.55, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3231, 2952 (N-H), 1703 (ester C=O), 1681 (amide C=O), 1520 (N-H); δ_H (400 MHz, DMSO-d_6) 8.92 (1H, d, *J* 7.0, N*H*CH), 8.41 - 8.24 (3H, m, CHN*H*$_3$), 7.44 - 7.28 (6H, m, 5 x Ar C*H*, N*H*CH_2), 5.02 (2H, s, C*H*$_2$Ph), 4.28 (1H, ddd, *J* 8.5, 7.5, 5.0 Hz, NHC*H*CH_2), 3.93 - 3.84 (1H, m, NHC*H*CH_3), 3.61 (3H, s, OC*H*$_3$), 3.02 (2H, q, *J* 5.5 Hz, NHC*H*$_2$), 1.83 - 1.58 (2H, m, CHC*H*$_2$), 1.58 - 1.43 (2H, m, C*H*$_2$), 1.39 (2H, d, *J* 7.0 Hz, CHC*H*$_3$); δ_C (100 MHz, DMSO-d_6) 172.0 (CH*C*ONH), 170.0 (*C*O_2Me), 156.1 (*C*O_2Bn), 137.2 (*ipso C*), 128.1, 127.7, 127.3 (*ortho*, *meta* and *para C*H), 65.1 (*C*H_2Ph), 51.4 (O*C*H_3, *C*HCH_2), 48.5 (*C*HCH_3), 38.0 (NH*C*H_2), 27.8, 23.3 (2 x *C*H_2) 17.2 (CH*C*H_3); m/z (ESI+) 352.2 ([M-Cl]+, 100%), 374.1 ([M-HCl+Na]+, 7%).

α-*N*-(*N*-Acetyl-L-alanyl)-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester 2.15



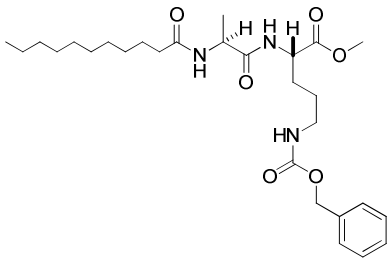
α-*N*-(L-Alanyl)-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester hydrochloride **2.14** (1.00 g, 2.52 mmol) and triethylamine (0.70 mL, 5.05 mmol) were stirred on ice in CH_2Cl_2 (20 mL). Acetic anhydride (0.27 g, 2.78 mmol) was added slowly to give a colourless solution. This was stirred to room temperature over 2 hours. Water (20 mL) was added and the phases separated. The organic phase was washed with citric acid solution (10% w/w, 2 x 20 mL) and saturated aqueous $NaHCO_3$ solution (2 x 20 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give **2.15** as a white crystalline solid (0.73 g, 1.83 mmol, 74 %); m.p. 153 - 154ºC; $[\alpha]_D^{33}$ -2.1 (c = 1.0, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3291 (N-H), 3066 (aromatic C-H), 2951 (aliphatic C-H), 1736 (ester C=O), 1681, 1631 (amide C=O), 1536 (N-H); δ_H (400 MHz, $CDCl_3$) 7.45 - 7.26 (6H, m, Ar C*H*, N*H*CHCH$_2$), 6.69 (1H, d, *J* 7.5 Hz, N*H*CHCH$_3$), 5.48 - 5.33 (1H, m, N*H*CH$_2$), 5.09 (2H, s, C*H*$_2$Ph), 4.66 - 4.49 (2H, m, NHC*H*CH$_3$, NHC*H*CH$_2$), 3.73 (3H, s, OC*H*$_3$), 3.18 (2H, q, *J* 6.5 Hz, NHC*H*$_2$), 1.96 (3H, s, COC*H*$_3$), 1.88 (1H, m, NHCHC*H*$_2$), 1.70 (1H, m, NHCHC*H*$_2$), 1.62 - 1.44 (2H, m, NHCH$_2$C*H*$_2$), 1.37 (3H, d, *J* 6.5 Hz, CHC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 172.7, 172.4, 170.3 (2 x *C*ONH, *C*O$_2$Me), 156.7 (*C*O$_2$Bn), 136.6 (*ipso C*), 128.5, 128.1 (*ortho*, *meta* and *para C*H), 66.7 (*C*H$_2$Ph), 52.5 (CO*C*H$_3$), 52.1 (*C*HCH$_3$), 48.9 (NH*C*HCH$_2$), 40.4 (NH*C*H$_2$), 28.9 (NHCH*C*H$_2$), 25.9 (NHCH$_2$*C*H$_2$), 23.0 (*C*H$_3$CO), 18.4 (CH*C*H$_3$); m/z (ESI+) 416.1 ([M+Na]+, 100.0%); HR-ESIMS: calculated for $C_{19}H_{27}N_3O_6Na$: 416.1792, found: 416.1790 $[M+Na]^+$.

(3*S*)-3-(Acetyl-L-alanyl)-aminopiperidine-2-one 2.02



10% palladium on activated carbon (~10 mg) was added to a solution of α-*N*-(*N*-acetyl-L-alanyl)-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester **2.15** (4.01 g, 10.2 mmol) in MeOH (25 mL). The reaction mixture was purged with hydrogen and stirred at 40°C under 1 atm pressure of hydrogen for 5 days. The reaction was filtered over celite and concentrated *in vacuo* to give a sticky white solid. Recrystallisation ($CHCl_3$:Et_2O) gave the lactam **2.02** as a white powdery solid (1.76 g, 7.74mmol, 76%); m.p. 210-210°C; $[\alpha]_D^{28}$ -38.8 (c = 1.0, MeOH); υ_{max}/cm^{-1} (neat) 3264 (N-H), 1625 (amide C=O), 1539 (N-H); δ_H (400 MHz, DMSO-d_6) 8.02 (1H, d, *J* 7.5 Hz, N*H*CHCH$_3$), 7.99 (1H, d, *J* 8.0 Hz, N*H*CHCH$_3$), 7.59 (1H, br. s., N*H*CH$_2$), 4.29 (1H, quin, *J* 7.5 Hz, NHC*H*CH$_3$), 4.13 (1H, ddd, *J* 11.0, 8.0, 6.0 Hz, NHC*H*CH$_2$), 3.14 - 3.00 (2H, m, NHC*H*$_2$), 1.99 - 1.91 (1H, m, lactam NHCHC*H*$_2$), 1.84 (3H, s, C*H*$_3$CO), 1.82 - 1.68 (2H, m, NHCH$_2$C*H*$_2$), 1.57 (1H, qd, *J* 11.5, 4.5 Hz, NHCHC*H*$_2$), 1.20 (3H, d, *J* 7.5 Hz, CHC*H*$_3$); δ_C (100 MHz, DMSO-d_6) 172.1, 169.7 (2 x *C*ONH), 168.8 (*C*ONHCH$_2$), 48.8 (NH*C*HCH$_2$), 47.9 (NH*C*HCH$_3$), 40.9 (NH*C*H$_2$), 27.5 (NHCH*C*H$_2$), 22.5 (CH$_3$CO), 21.0 (NHCH$_2$*C*H$_2$), 18.5 (CH*C*H$_3$); m/z (ESI+) 249.8 ([M+H]+, 100.0%); HR-ESIMS: calculated for $C_{10}H_{17}N_3O_3Na$: 250.1162, found: 250.1162 $[M+Na]^+$.

α-*N*-(*N*-Undecanoyl-L-alanyl)-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester 2.16

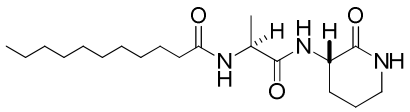


Undecanoic acid (3.0 g, 16.3 mmol) and DMF (3 drops) were stirred on ice in CH_2Cl_2 (50 mL). Oxalyl chloride (4.13 g, 32.2 mmol) was added dropwise with exotherm. The reaction was allowed to reach room temperature over 1 hour. The product was obtained by concentration *in vacuo* to give an orange oil which was used without further purification (3.37 g, 16.5 mmol, 100%); υ_{max}/cm^{-1} 2923 (C-H), 1801 (C=O); δ_H (400 MHz, $CDCl_3$) 2.90 (2H, t, *J* 7.5 Hz, C*H*$_2$CO), 1.73 (2H, quin, *J* 7.5 Hz, C*H*$_2$CH$_2$CO), 1.42 - 1.26 (14H, m, 7 x C*H*$_2$), 0.91 (3H, t, *J* 7.0 Hz, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 179.8 (*C*OCl), 47.1 (*C*H$_2$COCl), 31.9 (*C*H$_2$CH$_2$COCl), 29.5, 29.3, 29.1, 28.5, 28.4 25.1, 22.7 (7 x *C*H$_2$), 14.1 (*C*H$_3$); m/z (ESI+) 169.2 ([M-Cl]+, 10.3%); HR-ESIMS: calculated for $C_{11}H_{21}O$: 169.1587, found: 169.1601 $[M\text{-}Cl]^+$. Data are consistent to that previously reported.[6]

α-*N*-(L-Alanyl)-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester hydrochloride **2.14** (1.00 g, 2.52 mmol) and triethylamine (0.70 mL, 5.05 mmol) were stirred on ice in CH_2Cl_2 (20 mL). Undecanoyl chloride (0.52 g, 2.52 mmol) was added slowly to give a white solid. This was broken up with the addition of CH_2Cl_2 (10 mL) and the resulting suspension was stirred to room temperature over 2 hour affording a pale yellow suspension. Water (20 mL) was added and the phases were separated. The organic phase was washed with citric acid solution (10% w/w, 2 x 20 mL) and saturated aqueous $NaHCO_3$ solution (2 x 20mL), dried over Na_2SO_4, and concentrated *in vacuo* to give **2.16** as a white crystalline solid (1.09 g, 2.10 mmol, 83 %); m.p. 116 - 118 ºC; $[\alpha]_D^{33}$ -11.2 (c = 1.0, $CHCl_3$); υ_{max}/cm^{-1} 3307 (N-H), 1739

(ester C=O), 1691 (amide C=O), 1612 (N-H); δ_H (400 MHz, $CDCl_3$) 7.47 - 7.21 (5H, m, 5 x Ar C*H*), 6.91 (1H, d, *J* 7.5 Hz, N*H*CHCH$_3$), 6.18 (1H, d, *J* 7.5 Hz, N*H*CHCH$_2$), 5.08 (1H, br. s., N*H*CH$_2$), 5.02 (2H, s, C*H*$_2$Ph), 4.62 - 4.40 (2H, m, NHC*H*CH$_3$, NHC*H*CH$_2$), 3.66 (3H, s, OC*H*$_3$), 3.12 (2H, q, *J* 6.5 Hz, NHC*H*$_2$), 2.10 (2H, t, *J* 8.0 Hz, C*H*$_2$CO), 1.90 - 1.69 (1H, m, NHCHC*H*$_2$), 1.71 - 1.39 (5H, m, NHCHC*H*$_2$, NHCH$_2$C*H*$_2$, C*H*$_2$CH$_2$CO), 1.30 (3H, d, *J* 7.0 Hz, CHC*H*$_3$), 1.26 - 1.08 (14H, m, 7 x C*H*$_2$), 0.83 - 0.79 (3H, m, CH$_2$C*H*$_3$); δ_C (400 MHz, $CDCl_3$) 173.3, 172.4, 172.3 (2 x *C*ONH, *C*O$_2$Me), 156.6 (*C*O$_2$Bn), 136.6 (*ipso C*), 128.5, 128.1 (*ortho*, *meta* and *para C*H), 66.7 (*C*H$_2$Ph), 52.5, 52.0, 48.7 (*C*HCH$_3$, *C*HCH$_2$, OCH$_3$), 40.4 (NH*C*H$_2$), 36.5 (*C*H$_2$CO), 31.9, 29.6, 29.5, 29.4, 29.3, 29.0, 25.9, 25.6, 22.7 (9 x *C*H$_2$), 18.2 (CH*C*H$_3$), 14.2 (CH$_2$*C*H$_3$); m/z (ESI+) 542.3 ([M+Na]+, 100%), 281.1 ([M-($C_{14}H_{23}NO_2$)+, 17.9%), 520.2 ([M+H]+, 5.1%); HR-ESIMS: calculated for $C_{28}H_{45}N_3O_6Na$: 169.1587, found: 542.3194 $[M+Na]^+$.

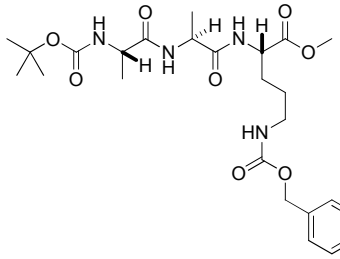
(3*S*)-3-(Undecanoyl-L-alanyl)-aminopiperidine-2-one 2.01



10% Palladium on activated carbon (~10 mg) was added to a solution of α-*N*-(*N*-undecanoyl-L-alanyl)-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester **2.16** (0.50 g, 0.96 mmol) in MeOH (20 mL). The reaction mixture was purged with hydrogen and stirred at room temperature under a constant 1 atm pressure hydrogen for 48 hours. The reaction was filtered over celite and concentrated *in vacuo* to give a sticky white solid. Recrystallisation ($CHCl_3$:Et_2O) afforded the lactam **2.01** as a white powdery solid (0.10 g, 0.31 mmol, 32 %); mp 179 - 180°C; $[\alpha]_D^{28}$ +17.3 (c = 1.11, MeOH); υ_{max}/cm^{-1} (neat) 3294, 3242 (N-H), 1683 (amide C=O), 1631 (N-H); δ_H (400 MHz, DMSO-d_6) 7.94 (2H, m, N*H*CHCH$_2$, N*H*CHCH$_3$), 7.59 (1H, br. s., N*H*CH$_2$), 4.29 (1H, quin., *J* 7.5 Hz, NHC*H*CH$_3$), 4.20 - 4.05 (1H, m, NHC*H*CH$_3$), 3.14 (2H, br. s., NHC*H*$_2$), 2.10 (2H, t, *J* 7.5 Hz, C*H*$_2$CONH), 2.03 - 1.93 (1H, m, NHCHC*H*$_2$), 1.83 - 1.65 (2H, m, NHCH$_2$C*H*$_2$), 1.65 - 1.53 (1H, m, NHCHC*H*$_2$), 1.52

- 1.42 (2H, m, CH_2CH$_2$CO), 1.25 (14H, m, 7 x CH_2), 1.20 (3H, d, *J* 7.0 Hz, CHCH_3), 0.91 - 0.83 (3H, m, CH$_2$CH_3); δ_C (100 MHz, DMSO-d$_6$) 172.1, 171.8, (2 x *C*ONH), 169.7 (*C*ONHCH$_2$), 48.8 (*C*HCH$_3$), 47.8 (NH*C*HCH$_2$), 40.9 (NH*C*H$_2$), 35.1 (CO*C*H$_2$), 31.3, 29.0, 28.9, 28.7, 28.6, 25.2, 22.1, 20.9 (9 x *C*H$_2$), 18.3 (CH*C*H$_3$), 14.0 (CH$_2$*C*H$_3$); m/z (ESI+) 354.2 ([M+H]+, 100%); HR-ESIMS: calculated for $C_{19}H_{35}N_3O_3Na$: 376.2570, found: 376.2569 $[M+Na]^+$.

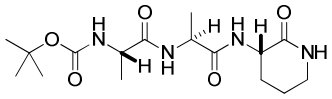
α-*N*-[(*N*-*tert*-Butoxycarbonyl-L-alanyl)-L-alanyl]-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester 2.17



N-*tert*-Butoxycarbonyl-L-alanine (0.87 g, 4.6 mmol) and HATU (1.74 g, 4.6 mmol) were stirred in CH_2Cl_2 (20 mL). After 5 minutes, α-*N*-(L-alanyl)-δ-N-(benzyloxycarbonyl)-L-ornithine methyl ester hydrochloride **2.14** (1.82 g, 4.6 mmol) was added and the suspension cooled to 0 °C using an ice-salt bath. The mixture was stirred at 0 °C for 10 minutes and triethylamine (1.9 mL, 13.7 mmol) was added slowly. The yellow suspension was stirred at room temperature until the reaction mixture became a clear yellow solution (3 hours) indicating completion. pH 2 buffer (20 mL) was added and the phases separated. The organic layer was washed with pH 2 buffer (20 mL), aqueous saturated $NaHCO_3$ solution (2 x 20 mL) and water (20 mL). The organic phase was concentrated *in vacuo* and the yellow residue taken into toluene. pH 2 buffer (20 mL) was added and the toluene layer was washed as before. The organic phase was concentrated *in vacuo* and dried by toluene azeotrope (4 x 20 mL). Residual solvent was removed under vacuum to afford **2.17** as a white crystalline solid (2.71 g; 5.1 mmol; 74 %); m.p. 119 - 120ºC; $[\alpha]_D^{33}$ -17.6 (c =1.0, $CHCl_3$); υ_{max}/cm^{-1} 3304 (N-H), 2972 (aromatic C-H), 2931 (aliphatic C-H),

1735 (ester C=O), 1689, 1636 (amide C=O), 1517 (N-H); δ_H (400 MHz, DMSO-d_6) 8.25 (1H, d, *J* 7.5 Hz, N*H*CHCH$_2$), 7.82 (1H, d, J 7.5 Hz, CON*H*CHCH$_3$), 7.40 - 7.29 (5H, m, 5 x Ar C*H*), 7.27 (1H, t, *J* 5.5 Hz, N*H*CH$_2$), 6.95 (1H, d, *J* 7.5 Hz, ^{t}BuCO$_2$N*H*), 5.02 (2H, s, C*H*$_2$Ph), 4.34 - 4.25 (1H, m, NHC*H*CH$_3$), 4.20 (1H, ddd, *J* 8.5, 7.5, 5.0 Hz, NHC*H*CH$_2$), 3.99 - 3.90 (1H, m, ^{t}BuCO$_2$NHC*H*), 3.62 (3H, s, OC*H*$_3$), 3.00 (2H, q, *J* 6.0 Hz, NHC*H*$_2$), 1.79 - 1.65 (1H, m, NHCHC*H*$_2$), 1.65 - 1.52 (1H, m, NHCHC*H*$_2$), 1.51 - 1.32 (11H, m, NHCH$_2$C*H*$_2$, C(C*H*$_3$)$_3$), 1.21 (3H, d, *J* 7.0 Hz, CONHCHC*H*$_3$), 1.16 (3H, d, *J* 7.5 Hz, ^{t}BuCO$_2$NHCHC*H*$_3$); δ_C (100 MHz, DMSO-d_6) 172.3, 172.2 (2 x *C*ONH, *C*O$_2$Me), 156.0, 155.0 (*C*O$_2$Bn, *C*O$_2^t$Bu), 137.2 (*ipso C*), 128.3, 127.7 (*ortho*, *meta* and *para C*H), 78.0 (*C*(CH$_3$)$_3$), 65.1 (*C*H$_2$Ph), 51.8, 51.7 (O*C*H$_3$, NH*C*HCH$_2$), 49.5 (^{t}BuCO$_2$NH*C*H), 47.6 (CONH*C*HCH$_3$), 40.2 (NH*C*H$_2$), 28.2 (C(*C*H$_3$)$_3$), 28.1 (NHCH*C*H$_2$), 25.8 (NHCH$_2$CH$_2$), 18.3 (CHCH$_3$), 18.0 (CHCH$_3$); m/z (ESI+) 545.2 ([M+Na]+, 100.0%), 445.2 ([M-Ph], 9.6%); HR-ESIMS: calculated for $C_{25}H_{38}N_4O_8Na$: 545.2582, found: 545.2587 $[M+Na]^+$.

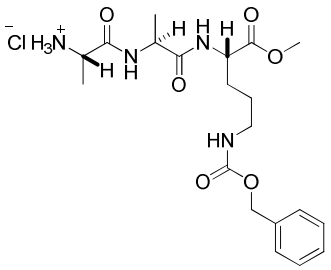
(3*S*)-3-[(*N*-*tert*-Butoxycarbonyl-L-alanyl) -L-alanyl]-aminopiperidine-2-one 2.08



10% Palladium on activated carbon (10 mg) was added to a solution of α-*N*-(*N*-*tert*-butoxycarbonyl-L-alanyl-L-alanyl)-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester **2.17** (1.00 g, 1.91 mmol) in MeOH (10 mL). The reaction mixture was purged with hydrogen and stirred at 40°C under 1 atm of hydrogen for 2 days. The reaction was filtered over celite and concentrated *in vacuo* to give a sticky off-white solid. This was purified by reverse phase HPLC and lyophilisation to give the peptide as a bright white solid (0.065 g, 0.182 mmol, 10 %); Decomposed 220 - 222°C; $[\alpha]_D^{28}$ +17.3 (c = 1.13, MeOH); υ_{max}/cm^{-1} (neat) 3300 (N-H), 1659 (amide C=O) , 1571 (N-H); δ_H (700 MHz, CD$_3$OD) 4.37 (1H, q, *J* 7.0 Hz, CONHC*H*CH$_3$), 4.31 - 4.13 (1H, m, NHC*H*CH$_2$), 4.05 (1H, m, ^{t}BuCO$_2$NHC*H*), 3.30 - 3.27 (2H, m, NHC*H*$_2$), 2.16 - 2.04 (1H, m, NHCHC*H*$_2$), 1.96 - 1.89 (1H, m, NHCH$_2$C*H*$_2$), 1.89 - 1.59 (2H, m,

NHCHCH_2, NHCH$_2$CH_2), 1.44 (9H, s, C(CH_3)$_3$), 1.38 (3H, d, *J* 7.0 Hz, CONHCHCH_3), 1.31 (3H, d, *J* 7.5 Hz, ^{t}BuCO$_2$NHCHCH_3); δ_C (175 MHz, CD_3OD) 173.1, 172.2, 170.1 (3 x *C*ONH), 155.7 (*C*O$_2^t$Bu), 78.5 (*C*(CH$_3$)$_3$, 49.5 (CONH*C*HCH$_3$), 48.9 (NH*C*HCH$_2$), 47.8 (^{t}BuCO$_2$NH*C*H), 40.2 (NH*C*H$_2$), 26.6 (C(*C*H$_3$)$_3$), 26.3 (NHCH*C*H$_2$), 20.1 (NHCH$_2$*C*H$_2$), 15.9, 15.9 (2 x CH*C*H$_3$); m/z (ESI+) 379.2 ([M+Na]+, 100.0%); HR-ESIMS: calculated for $C_{16}H_{28}N_4O_5Na$: 379.1952, found: 379.1951 $[M+Na]^+$.

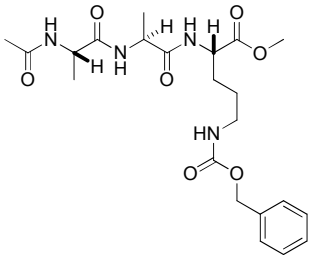
α-*N*-(L-Alanyl-L-alanyl)-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester hydrochloride 2.18



α-*N*-[(*N*-*tert*-Butoxycarbonyl-L-alanyl)-L-alanyl]-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester **2.17** (6.70 g, 12.8 mmol) was deprotected by stirring in methanolic HCl (2M, 25.0 mL) for 3 hours at room temperature. Concentration *in vacuo* gave the crude product as a sticky brown oil. Recrystallisation (MeOH:Et$_2$O) afforded **2.18** as a white crystalline solid (5.20 g, 11.4 mmol, 89 %); m.p. >300ºC; υ_{max}/cm^{-1} 3278 (br. NH$_3$), 3050 (aromatic C-H), 2951 (aliphatic C-H), 1735 (ester C=O), 1655 (amide C=O), 1560 (N-H); δ_H (400 MHz, DMSO-d$_6$) 8.68 (1H, d, *J* 7.5 Hz, N*H*CHCH$_3$), 8.41 (1H, d, *J* 7.0 Hz, N*H*CHCH$_2$), 8.30 (3H, br. s., N*H*$_3$CH), 7.46 - 7.23 (5H, m, 5 x Ar C*H*), 5.02 (2H, s, C*H*$_2$Ph), 4.37 (1H, quin, *J* 7.0 Hz, NHC*H*CH$_3$), 4.25 - 4.18 (1H, m, NHC*H*CH$_2$), 3.90 - 3.82 (1H, m, NH$_3$C*H*), 3.60 (3H, s, OC*H*$_3$), 3.00 (2H, q, *J* 6.5 Hz, NHC*H*$_2$), 1.82 - 1.55 (2H, m, NHCHC*H*$_2$), 1.55 - 1.38 (2H, m, NHCH$_2$C*H*$_2$), 1.36 (3H, d, *J* 7.0 Hz, NHCHC*H*$_3$), 1.27 (3H, d, *J* 7.0 Hz, NHCHC*H*$_3$); δ_C (100 MHz, DMSO-d$_6$) 172.3, 172.0, 169.0 (2 x *C*ONH, *C*O$_2$Me), 156.1 (*C*O$_2$Bn), 137.2 (*ipso C*), 128.3, 127.7 (*ortho*, *meta* and *para C*H),

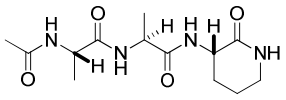
65.1 (*C*CH_2Ph), 51.8, 48.1, 47.9 (O*C*H_3, NH*C*HCH_3, NH_3*C*HCH_3, NH*C*HCH_2), 47.9 (NH*C*H_2), 29.7, 27.3 (2 x *C*H_2), 18.0 (CH*C*H_3), 17.7 (CH*C*H_3).

α-*N*-[(*N*-Acetyl-L-alanyl)-L-alanyl]-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester 2.19



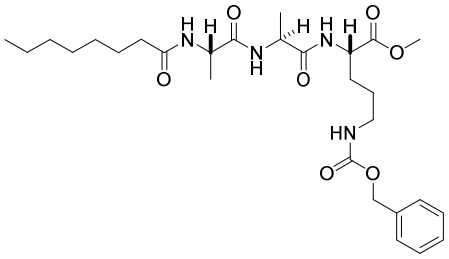
Acetic anhydride (0.26 g, 2.6 mmol) was added slowly to a cooled and stirred solution of **2.18** (1.0 g, 2.4 mmol) and triethylamine (0.77 g, 5.5 mmol) in CH_2Cl_2 (20 mL). The reaction was allowed to reach room temperature overnight. pH 2 buffer was added and the phases separated. The organic phase was further washed with saturated aqueous $NaHCO_3$ (20 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give **2.19** as a bright white solid (0.50 g, 1.3 mmol, 50 %); m.p. 200 - 201ºC; $[\alpha]_D^{27}$ -39.6 (c = 0.35, MeOH); υ $_{max}$/cm^{-1} 3301 (N-H), 2971 (Ar C-H), 2901 (aliphatic C-H), 1736 (ester C=O), 1689 (amide C=O), 1626 (amide C=O), 1533 (N-H); δ_H (400 MHz, DMSO-d_6) 8.14 (1H, d, *J* 7.5 Hz, N*H*CH), 8.04 (1H, d, *J* 7.5 Hz, N*H*CH), 7.96 (1H, d, *J* 7.5 Hz, N*H*CH), 7.43 - 7.30 (m, 5H, 5 x Ar C*H*), 7.27 (1H, t, *J* 5.5 Hz, N*H*CH_2), 5.02 (2H, s, CH_2Ph), 4.36 - 4.15 (3H, m, NHC*H*CH_2, 2 x NHC*H*CH_3), 3.62 (3H, s, OCH_3), 3.01 (2H, q, *J* 6.5 Hz, NHCH_2), 1.84 (3H, s, CH_3CO), 1.79 - 1.66 (1H, m, NHCHCH_2), 1.65 - 1.55 (1H, m, NHCHCH_2), 1.53 - 1.38 (2H, m, $NHCH_2$CH_2), 1.22 (2H, d, *J* 7.0 Hz, CHCH_3), 1.18 (3H, d, *J* 7.0 Hz, CHCH_3); δ_C (100 MHz, DMSO-d_6) 172.3 (2 x *C*ONHCHCH_3), 172.0 (*C*O_2Me), 169.1 (CH_3*C*O), 156.1 (*C*O_2Ph), 137.2 (*ipso C*), 128.3, 127.7 (*ortho*, *meta* and *para C*H), 65.1 (CH_2Ph), 51.8, 51.7 (O*C*H_3, NH*C*HCH_2) 48.1, 47.7 (2 x *C*HCH_3), 40.1 (NH*C*H_2), 28.1 (NHCH*C*H_2), 25.8 ($NHCH_2$*C*H_2), 22.5 (*C*H_3CO), 18.1 (2 x CH*C*H_3); m/z (ESI+) 487.1 ([M+Na]+, 100.0%); HR-ESIMS: calculated for $C_{22}H_{32}N_4O_7Na$: 487.2163, found: 487.2165 $[M+Na]^+$.

(3*S*)-3-[(*N*-Acyl-L-alanyl)-L-alanyl]-aminopiperidine-2-one 2.04



10% Palladium on activated carbon (~20 mg) was added to a solution of **2.19** (1.20 g, 2.58 mmol) in MeOH (50 mL). The reaction mixture was purged with hydrogen and stirred at 40°C under 1 atm of hydrogen for 72 hours. The reaction mixture was filtered over celite and the filtrate concentrated *in vacuo* to give a powdery white solid (0.55 g, 1.9 mmol, 74 %); $[\alpha]_D^{25}$ -60.6 (c = 0.18, MeOH); m.p. 183 - 185°C (Decomposed); υ_{max}/cm^{-1} (neat) 3301 (N-H), 2934 (aliphatic C-H), 1659, 1638 (Amide C=O); δ_H (400 MHz, DMSO-d_6) 8.03 (1H, d, *J* 7.0 Hz, *N*HCHCH$_3$), 7.94 (1H, d, *J* 7.0 Hz, N*H*CHCH$_3$), 7.89 (1H, d, *J* 8.0 Hz, N*H*CHCH$_2$), 7.60 - 7.56 (1H, m, N*H*CH$_2$), 4.30 - 4.19 (2H, m, 2 x NHC*H*CH$_3$), 4.10 (1H, ddd, *J* 10.5, 7.5, 6.0 Hz, NHC*H*CH$_2$), 3.15 - 3.06 (2H, m, NHC*H*$_2$), 2.02 - 1.93 (1H, m, NHCHC*H*$_2$), 1.82 (3H, s, C*H*$_3$CO), 1.81 - 1.65 (2H, NHCH$_2$C*H*$_2$), 1.55 (1H, br. qd, *J* 11.5, 4.5 Hz, NHCHC*H*$_2$), 1.21 (3H, d, *J* 7.0 Hz, CHC*H*$_3$), 1.18 (3H, d, *J* 7.0 Hz, CHC*H*$_3$); δ_C (100 MHz, DMSO-d_6) 171.9, 171.7 (2 x *C*ONH), 169.6 (CH$_3$*C*O), 169.0 (*C*ONHCH$_2$), 49.4, 48.5 (2 x NH*C*HCH$_3$, NH*C*HCH$_2$), 40.8 (NH*C*H$_2$), 27.4 (NHCH*C*H$_2$), 22.5 (*C*H$_3$CO), 20.9 (NHCH$_2$*C*H$_2$), 18.3, 18.1 (2 x CH*C*H$_3$); m/z (ESI+) 321.1 ([M+Na]+, 100.0%); HR-ESIMS: calculated for $C_{13}H_{22}N_4O_4Na$: 321.1533, found: 321.1531 $[M+Na]^+$.

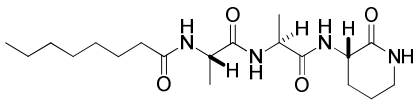
α-*N*-[(*N*-Octanoyl-L-alanyl)-L-alanyl]-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester 2.20



Octanoyl chloride (0.42 g, 2.60 mmol) was added slowly to a cooled and stirred solution of **2.18** (1.0 g, 2.36 mmol) and triethylamine (0.52 g, 5.2 mmol) in CH_2Cl_2 (20 mL). The reaction was allowed to reach room temperature overnight. pH 2 buffer was added and the phases were separated. The organic phase was further washed with saturated aqueous $NaHCO_3$ (20 mL) and concentrated *in vacuo* to give a yellow waxy solid. This was suspended in boiling toluene and cooled with stirring before washing with pH 2 buffer (2 x 20mL), saturated aqueous $NaHCO_3$ (20 mL) and water (20 mL). Concentration of the organic phase followed by toluene azeotrope (2 x 20 mL) afforded the product as a white solid (0.87 g, 1.59 mmol, 67%); m.p. 175-176ºC; $[\alpha]_D^{25}$ -54.1 (c = 0.13, MeOH); υ_{max}/cm^{-1} (neat) 3292 (N-H), 1736 (ester C=O), 1689, (amide C=O), 1534 (N-H); δ_H (500 MHz, DMSO-d_6) 8.16 (1H, d, *J* 7.5 Hz, N*H*CHCH$_3$), 7.94 (1H, d, *J* 7.0 Hz, N*H*CHCH$_2$), 7.88 (1H, d, *J* 7.5 Hz, N*H*CHCH$_3$), 7.42 - 7.30 (5H, m, 5 x Ar C*H*), 7.25 (1H, t, *J* 5.5 Hz, N*H*CH$_2$), 5.01 (2H, s, C*H*$_2$Ph), 4.32 - 4.17 (3H, m, 2 x NHC*H*CH$_3$, NHC*H*CH$_2$), 3.61 (3H, s, OC*H*$_3$), 3.04 - 2.96 (2H, m, NHC*H*$_2$), 2.09 (2H, t, *J* 7.5 Hz, C*H*$_2$CONH), 1.79 - 1.54 (2H, m, NHCHC*H*$_2$), 1.52 - 1.39 (4H, m, NHCH$_2$C*H*$_2$, C*H*$_2$CH$_2$CONH), 1.29 - 1.22 (8H, m, 4 x C*H*$_2$), 1.20 (3H, d, *J* 7.5 Hz, CHC*H*$_3$), 1.19 (3H, d, *J* 7.0 Hz, CHC*H*$_3$), 0.85 (3H, t, *J* 7.5 Hz, CH$_2$C*H*$_3$); δ_C (125 MHz, DMSO-d_6) 172.8, 172.6, 172.5 (4 x *C*ONH), 156.6 (*C*O$_2$Bn), 137.7 (*ipso C*), 128.8, 128.2 (*ortho*, *meta* and *para C*H), 65.6 (*C*H$_2$Ph), 52.3 (O*C*H$_3$), 52.2 (NH*C*HCH$_2$), 48.4, 48.2 (2 x NHCHCH$_3$), 40.4 (NH*C*H$_2$), 35.6 (*C*H$_2$CONH), 31.6, 29.1, 28.9, 28.6, 26.3, 25.7, 22.5 (7 x *C*H$_2$), 18.6

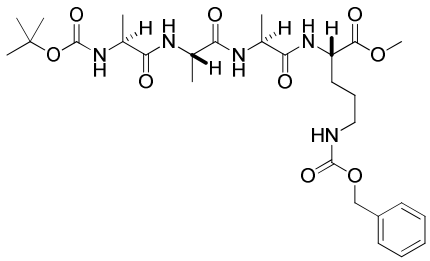
(CH*C*H$_3$), 18.5 (CH*C*H$_3$), 14.4 (CH$_2$*C*H$_3$); m/z (ESI+) 571.3 ([M+Na]+, 100.0%); HR-ESIMS: calculated for $C_{28}H_{44}N_4O_7Na$: 571.3102, found: 571.3102 $[M+Na]^+$.

(3*S*)-3-[(*N*-Octanoyl-L-alanyl)-L-alanyl]-aminopiperidine-2-one 2.03



10% Palladium on activated carbon (~5 mg) was added to a solution of **2.20** (0.18 g, 0.33 mmol) in MeOH (25 mL). The reaction mixture was purged with hydrogen and stirred at 40°C under a 1 atm of hydrogen for 2 days. The reaction was filtered over celite and concentrated *in vacuo* to give a sticky off-white solid. This was purified by reverse phase HPLC and lyophilisation to give lactam **2.03** as a bright white solid (0.025 g, 0.065 mmol, 20 %); Decomposed 196 - 198 °C; $[\alpha]_D^{28}$ -4.32 (c = 0.10, $CHCl_3$); υ_{max}/cm^{-1} 3287 (N-H), 1628 (amide C=O), 1537 (N-H); δ_H (600 MHz, DMSO-d_6) 7.96 (1H, d, *J* 7.5 Hz, N*H*CHCH$_3$), 7.92 (1H, d, *J* 7.5 Hz, N*H*CHCH$_2$), 7.87 (1H, d, *J* 7.5 Hz, N*H*CHCH$_3$), 7.58 (1H, s, N*H*CH$_2$), 4.29 - 4.20 (2H, m, 2 x NHC*H*CH$_3$), 4.13 - 4.08 (1H, m, NHC*H*CH$_2$), 3.13 - 3.09 (2H, m, NHC*H*$_2$), 2.08 (2H, t, *J* 7.0 Hz, C*H*$_2$CONH), 2.01 - 1.94 (1H, m, NHCHC*H*$_2$), 1.81 - 1.66 (2H, m, NHCH$_2$C*H*$_2$), 1.59 - 1.50 (1H, m, NHCHC*H*$_2$), 1.49 - 1.42 (2H, m, C*H*$_2$CH$_2$CO), 1.31 - 1.19 (11H, m, 4 x C*H*$_2$, CHC*H*$_3$), 1.17 (3H, d, *J* 7.5 Hz, CHC*H*$_3$), 0.86 (3H, t, *J* 7.0 Hz, CH$_2$C*H*$_3$); δ_C (150 MHz, DMSO-d_6) 172.5, 172.4, 172.2, 170.1 (4 x *C*ONH), 49.3 (NH*C*HCH$_2$), 48.4 (2 x NH*C*HCH$_3$), 41.3 (NH*C*H$_2$), 35.6 (*C*H$_2$CO), 31.6, 39.1, 27.9, 25.7, 22.5, 21.4 (7 x *C*H$_2$), 18.9, 18.5 (2 x CH*C*H$_3$), 14.4 (CH$_2$*C*H$_3$); m/z (ESI+) 405.1 ([M+Na]+, 100.0%); HR-ESIMS: calculated for $C_{19}H_{34}N_4O_4Na$: 405.2472, found: 405.2475 $[M+Na]^+$.

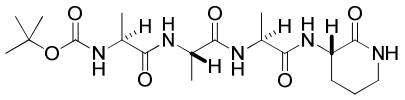
α-*N*-[(*N*-*tert*-Butoxycarbonyl-L-alanyl)-L-alanyl-L-alanyl]-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester 2.21



N-*tert*-Butoxycarbonyl-L-alanine (0.90 g, 4.7 mmol) and HATU (1.8 g, 4.7 mmol) were stirred in CH_2Cl_2 (30 mL). After 5 minutes **2.18** (1.8 g, 4.7 mmol) was added and the suspension cooled to 0 °C using an ice-salt bath. The mixture was stirred at 0 °C for 10 minutes and triethylamine (2.0 mL, 14.0 mmol) was added slowly. The yellow suspension was stirred to room temperature overnight to give a clear gelatinous yellow mixture. This was stirred with pH 2 buffer (20 mL) and the suspension was filtered. The resulting gel-like solid was washed with pH 2 buffer (2 x 20 mL), saturated aqueous $NaHCO_3$ (20 mL) and water (20 mL). The organic and aqueous filtrates were separated, and the organic phase combined with the filtercake. This mixture was concentrated *in vacuo* and the product obtained following toluene azeotrope (3 x 20 mL) as a white glassy solid (1.8 g, 3.0 mmol, 63 %); m.p. 98 - 100 ºC; $[\alpha]_D^{34}$ -28.4 (c = 0.18, MeOH); υ_{max}/cm^{-1} 3279 (N-H), 2978 (aromatic C-H), 2903 (aliphatic C-H), 1731 (ester C=O), 1701 (carbamate C=O), 1629 (amide C=O), 1519 (N-H); δ_H (400 MHz, DMSO-d_6) 8.18 (1H, d, *J* 7.5 Hz, N*H*CHCH$_2$), 7.94 (1H, d, *J* 7.5 Hz, N*H*CHCH$_3$), 7.87 (1H, d, *J* 7.0 Hz, N*H*CHCH$_3$), 7.51 - 7.30 (5H, m, 5 x Ar C*H*), 7.29 - 7.25 (1H, m, NHC*H*$_2$), 6.98 (1H, d, *J* 7.5 Hz, ^{t}BuCO$_2$N*H*), 5.02 (2H, s, C*H*$_2$Ph), 4.36 - 4.18 (3H, m, 2 x NHC*H*CH$_3$, NHC*H*CH$_2$), 3.96 (1H, quin., *J* 7.0 Hz, ^{t}BuCO$_2$NHC*H*), 3.62 (3H, s, OC*H*$_3$), 3.01 (2H, q, *J* 6.5 Hz, NHC*H*$_2$), 1.80 - 1.66 (1H, m, NHCHC*H*$_2$), 1.66 - 1.53 (1H, m, NHCHC*H*$_2$), 1.53 - 1.42 (2H, m, NHCH$_2$C*H*$_2$), 1.39 (9H, s, C(C*H*$_3$)$_3$), 1.26 - 1.12 (9H, m, 3 x CHC*H*$_3$); δ_C (100 MHz,

DMSO-d_6) 172.4, 172.3, 172.2 (3 x *C*ONH), 171.7 (CO_2CH_3), 156.1, 155.1 (*C*O_2Ph, *C*$O_2{}^t$Bu), 137.2 (*ipso C*), 128.9, 128.3, 127.7 (*ortho*, *meta* and *para C*H), 78.1 (*C*$(CH_3)_3$), 65.1 (*C*H_2Ph), 51.8, 51.7 (O*C*H_3, NH*C*H*C*H_3), 49.7 (^{t}BuCO_2NH*C*H), 47.9, 47.7 (2 x NH*C*HCH_3), 38.9 (NH*C*H_2), 28.2 (C(*C*$H_3)_3$), 28.1 (NHCH*C*H_2), 25.9 (NHCH_2*C*H_2), 18.1, 18.0 (3 x CH*C*H_3); m/z (ESI+) 616.1 ([M+Na]+, 100.0%); HR-ESIMS: calculated for $C_{28}H_{43}N_5O_9Na$: 616.2953, found: 616.2978 $[M+Na]^+$.

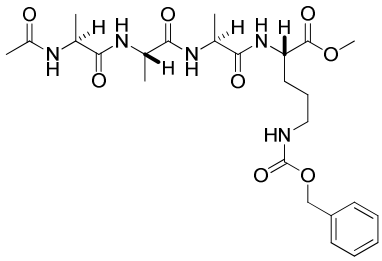
(3*S*)-3-[(*N*-*tert*-Butoxycarbonyl-L-alanyl)-L-alanyl-L-alanyl]-aminopiperidine-2-one 2.09



10% Palladium on activated carbon (~10 mg) was added to a solution of **2.21** (0.34 g, 0.57 mmol) in MeOH (20 mL). The reaction mixture was purged with hydrogen and stirred at 40°C under 1 atm of hydrogen for 72 hours. The reaction mixture was filtered over celite and the filtrate concentrated *in vacuo* to give the crude product. This was purified by reverse phase HPLC followed by lypophilisation to give **2.09** as bright white solid (0.023 g, 0.054 mol, 9 %); Decomposed at 255 - 257 °C; υ_{max}/cm^{-1} 3342, 3305 (N-H), 2953 (aliphatic C-H), 1683 (amide C=O); δ_H (700 MHz, DMSO-d_6) 7.93 - 7.89 (2H, m, ^{t}BuCO_2N*H*CH, N*H*CHCH_2), 7.84 (1H, d, *J* 7.5 Hz, N*H*CHCH_3), 7.58 - 7.56 (1H, m, N*H*CH_2), 6.94 (1H, d, *J* 7.5 Hz, NHC*H*CH_3), 4.31 - 4.17 (2H, m, 2 x NHC*H*CH_3), 4.11 (1H, ddd, J 11.0, 7.5, 6.0 Hz, NHC*H*CH_2), 3.97 - 3.91 (1H, m, , ^{t}BuCO_2NHC*H*), 3.15 - 3.09 (2H, m, NHC*H*$_2$), 2.01 - 1.95 (1H, m, NHCHC*H*$_2$), 1.83 - 1.65 (2H, m, NHCH$_2$C*H*$_2$), 1.54 (1H, dq, *J* 11.5, 4.5 Hz, NHCHC*H*$_2$), 1.37 (9H, s, C(C*H*$_3)_3$), 1.21 (3H, d, *J* 7.0 Hz, CHC*H*$_3$), 1.20 (3H, d, *J* 7.0 Hz, CHC*H*$_3$), 1.16 (3H, d, *J* 7.0 Hz, ^{t}BuCO_2NHCHC*H*$_3$); δ_C (175 MHz, DMSO-d_6) 172.8, 172.7, 172.1, 170.8 (4 x *C*ONH), 155.5 (*C*$O_2{}^t$Bu), 78.5 (*C*$(CH_3)_3$), 50.1 (^{t}BuCO_2NH*C*H), 49.3 (NH*C*HCH_2), 48.5, 48.4 (2 x NH*C*HCH_3), 41.3 (NH*C*H_2), 28.6 (C(*C*$H_3)_3$), 27.9 (NHCH*C*H_2), 21.4 (NHCH_2*C*H_2), 18.9, 18.8, 18.5 (3 x

CH*C*H$_3$); m/z (ESI+) 450.1 ([M+Na]+, 100%). HR-ESIMS: calculated for $C_{19}H_{33}N_5O_6Na$: 450.2323, found: 450.2325 $[M+Na]^+$.

α-*N*-[(*N*-Acetyl-L-alanyl)-L-alanyl-L-alanyl]-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester 2.23

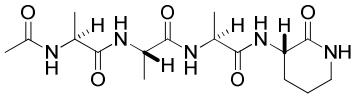


2.21 (1.95 g, 3.28 mmol) was deprotected by stirring in methanolic HCl (2M, 12.0 mL) for 3 hours at room temperature. Concentration *in vacuo* gave the crude deprotected product as a sticky brown oil, which was used immediately without further purification. $[\alpha]_D^{34}$ -54.7 (c = 0.11, MeOH);

A solution of the crude amine hydrochloride (3.28 mmol) and triethylamine (1.26 mL, 8.98 mmol) in THF (10 mL) and water (5 mL) was cooled to 0 - 5 °C. Acetic anhydride (0.51 mL, 5.39 mmol) was added dropwise and the reaction stirred at room temperature for 18 hours. pH 2 buffer was added, and the reaction was filtered. The filtercake was washed with water, and dried by toluene azeotrope to give **2.23** as a white solid (0.65 g, 1.21 mmol, 37%); m.p. 222 - 223 °C; $[\alpha]_D^{28}$ -62.3 (c = 0.49, MeOH); δ_H (400 MHz, DMSO-d_6) 8.25 (1H, d, *J* 7.5 Hz, N*H*CH), 8.15 - 8.11 (2H, m, 2 x N*H*CH), 8.07 (1H, d, *J* 7.5 Hz, N*H*CH), 7.42 - 7.28 (6H, m, 5 x Ar C*H*, N*H*CH_2), 5.02 (2H, s, CH_2Ph), 4.36 - 4.16 (4H, m, 4 x NHC*H*), 3.62 (3H, s, OCH_3), 3.06 - 2.96 (2H, m, NHCH_2), 1.78 - 1.54 (5H, m, CH_3CO, NHCHCH_2), 1.53 - 1.39 (2H, m, NHCH$_2$CH_2), 1.22 (3H, d, *J* 7.0 Hz, CHCH_3), 1.21 (d, *J* 7.5 Hz, CHCH_3), 1.19 (d, *J* 7.0 Hz, CHCH_3); δ_C (100 MHz, DMSO-d_6) 172.3, 172.2 (3 x *C*ONH), 171.5 (*C*O$_2$Me), 169.1 (CH$_3$*C*O), 156.1 (*C*O$_2$Ph), 137.2 (*ipso C*), 128.8, 128.3, 127.7 (*ortho*, *meta* and *para C*H), 65.1 (*C*H$_2$Ph), 51.8, 51.7 (NH*C*H, O*C*H$_3$), 48.2, 48.0, 47.9 (3 x NH*C*H), 40.1 (NH*C*H$_2$), 28.1 (NHCH*C*H$_2$),, 25.8 (NHCH$_2$*C*H$_2$), 22.5

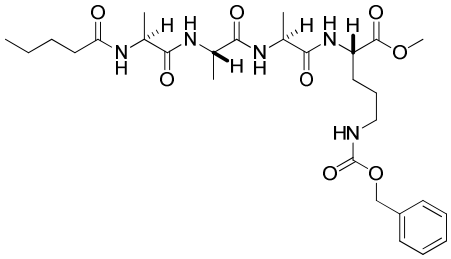
(*C*H_3CO), 18.1, 18.0, 17.9 (3 x CH*C*H_3); m/z (ESI+) 558.2 ([M+Na]+, 100.0%); HR-ESIMS: calculated for $C_{25}H_{37}N_5O_8Na$: 558.2534, found 558.2536 $[M+Na]^+$.

(3*S*)-[(*N*-Acetyl-L-alanyl)-L-alanyl-L-alanyl]-aminopiperidine-2-one 2.06



10% palladium on activated carbon (~20 mg) was added to a solution of **2.23** (0.65 g, 1.21 mmol) in MeOH (30 mL). The reaction mixture was purged with hydrogen and stirred at 40°C under 1 atm of hydrogen for 72 hours. The reaction mixture was filtered over celite and the filtrate concentrated *in vacuo* to give the crude product. This was purified by reverse phase HPLC followed by lypophilisation to give **2.06** as a bright white solid (0.11 g, 0.31 mmol, 26 %); m.p. 272 - 274 °C (decomposed); $[\alpha]_D^{26}$ -106.5 (c = 0.30, MeOH); δ_H (600 MHz, DMSO-d_6) 8.03 (1H, d, *J* 7.0 Hz, N*H*CHCH$_3$), 8.00 (1H, d, *J* 7.5 Hz, N*H*CHCH$_3$), 7.93 (1H, d, J 7.5 Hz, NHCHCH$_2$), 7.83 (1H, d, J 7.5 Hz, NHCHCH$_3$), 7.58 (1H, br. s., NHCH$_2$), 4.24 (3H, m, 3 x NHCHCH$_3$), 4.14 - 4.09 (1H, m, NHCHCH$_2$), 3.12 (2H, m, lactam NHCH$_2$), 2.01 - 1.93 (1H, m, NHCHCH$_2$), 1.82 (3H, s, COCH$_3$), 1.80 - 1.67 (2H, m, lactam CH$_2$), 1.53 (1H, br. qd, J 11.5, 4.5 Hz, NHCHCH$_2$), 1.21 (3H, d, J 7.5 Hz, CHCH$_3$), 1.20 (3H, d, J 7.0 Hz, CHCH$_3$), 1.17 (3H, d, J 7.0 Hz, CHCH$_3$); δ_C (150 MHz, DMSO-d_6) 172.6, 172.1, 172.1, 170.1, 169.5 (5 x *C*ONH), 49.3 (NH*C*HCH$_2$), 48.7, 48.5, 48.4 (3 x NH*C*H), 41.3 (NH*C*H$_2$), 28.0 (NHCH*C*H$_2$), 23.0 (*C*H$_3$CO), 21.4 (NHCH$_2$*C*H$_2$), 18.9, 18.6, 18.5 (3 x CH*C*H$_3$); m/z (ESI+) 392.1 ([M+Na]+, 100.0%); HR-ESIMS: calculated for $C_{16}H_{27}N_5O_5Na$: 392.1904, found 392.1910 $[M+Na]^+$.

α-*N*-[(*N*-Valeryl-L-alanyl)-L-alanyl-L-alanyl]-δ-*N*-(benzyloxycarbonyl)-L-ornithine methyl ester 2.24

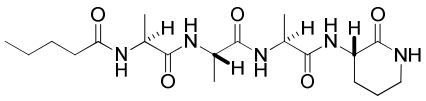


2.21 (0.867 g, 1.46 mmol) was deprotected by stirring in methanolic hydrochloric acid (2M, 6.0 mL) for 3 hours at room temperature. Concentration *in vacuo* gave the crude deprotected product as a sticky brown oil, which was used immediately without further purification.

A solution of the crude amine hydrochloride (1.46 mmol) and triethylamine (0.41 mL, 2.91 mmol) in THF (5 mL) and water (2 mL) was cooled to 0 - 5 °C. Valeryl chloride (0.17 mL, 1.46 mmol) was added dropwise and the reaction stirred at room temperature for 18 hours. pH 2 buffer was added, and the reaction was filtered. The aqueous and organic filtrates were separated, and the organic phase was combined with the filtercake. This was concentrated in vacuo, and dried by toluene azeotrope to give **2.24** as a white solid (0.82 g, 1.42 mmol, 98 %); m.p. 182 - 184 °C; $[\alpha]_D^{26}$ -32.9 (c = 0.2, MeOH); δ_H (700 MHz, DMSO-d_6) 8.19 (1H, d, *J* 7.5 Hz, N*H*CH), 8.02 (1H, d, *J* 7.0 Hz, N*H*CH), 7.96 (1H, d, *J* 7.5 Hz, N*H*CH), 7.90 (1H, d, *J* 7.5 Hz, N*H*CH), 7.36 - 7.28 (5H, m, Ar C*H*), 7.26 (1H, t, *J* 5.5 Hz, N*H*CH_2), 4.99 (2H, s, CH_2Ph), 4.32 - 4.16 (4H, m, NHC*H*CH_2, 3 x NHC*H*CH_3), 3.60 (3H, s, OCH_3), 3.03 - 2.94 (2H, m, NHCH_2), 2.11 - 2.07 (2H, q, *J* 8.0 Hz, NHCOCH_2), 1.73 - 1.67 (1H, m, NHCHCH_2), 1.62 - 1.56 (1H, m, NHCHCH_2), 1.48 - 1.40 (4H, m, CH_2, $NHCH_2CH_2$), 1.27 - 1.15 (11H, m, CH_2, 3 x CHCH_3), 0.86 - 0.82 (3H, m, CH_2CH_3); δ_C (175 MHz, DMSO-d_6) 172.8, 172.7, 172.4, 172.1 (4 x *C*ONH), 156.4 (*C*O_2Bn), 137.7 (*ipso C*), 128.8, 128.2, 128.1 (*ortho*, *meta* and *para C*H), 65.6 (*C*H_2Ph), 52.3, 52.2 (NH*C*HCH_2, O*C*H_3), 48.5, 48.3, 48.1 (3 x NH*C*HCH_3), 40.5 (NH*C*H_2), 35.2

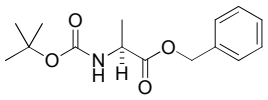
(CH_2CO), 28.5 ($NHCHCH_2$), 27.8, 26.3, 22.2 ($NHCH_2CH_2$, 2 x CH_2), 18.6, 18.4, 17.4 (3 x $CHCH_3$), 14.2 (CH_2CH_3); m/z (ESI+) 600.3 ([M+Na]+, 100.0%); HR-ESIMS: calculated for $C_{28}H_{43}N_5O_8Na$: 600.3004, found 600.3008 $[M+Na]^+$.

(3*S*)-[(*N*-Valeryl-L-alanyl)-L-alanyl-L-alanyl]-aminopiperidine-2-one 2.05



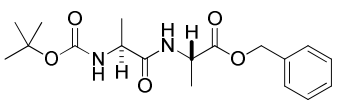
10% Palladium on activated carbon (~20 mg) was added to a solution of **2.24** (0.82 g, 1.42 mmol) in MeOH (30 mL). The reaction mixture was purged with hydrogen and stirred at 40 °C under 1 atm of hydrogen for 48 hours. The reaction mixture was filtered over celite and the filtrate concentrated *in vacuo* to give the crude product. This was purified by reverse phase HPLC and lyophilisation to give **2.05** as a bright white crystalline solid (0.03g, 0.07 mmol, 5 %); m.p. 167 - 169 °C; $[\alpha]_D^{26}$ -56.3 (c = 0.47, MeOH); δ_H (500 MHz, DMSO-d_6) 7.99 - 7.88 (4H, m, 3 x N*H*$CHCH_3$, N*H*$CHCH_2$), 7.59 - 7.55 (1H, m, N*H*CH_2), 4.34 - 4.20 (3H, m, 3 x $NHCHCH_3$), 4.15 - 4.08 (1H, m, $NHCHCH_2$), 3.15 - 3.09 (2H, m, $NHCH_2$), 2.10 (2H, t, *J* 7.5 Hz, CH_2CO), 2.03 - 1.94 (1H, m, $NHCHCH_2$), 1.83 - 1.66 (2H, m, 1H, $NHCH_2CH_2$), 1.65 - 1.50 (1H, m, $NHCHCH_2$), 1.50 - 1.42 (2H, m, CH_2CH_2CO), 1.30 - 1.18 (11H, m, 3 x $CHCH_3$, CH_2), 0.86 (3H, t, *J* 7.5 Hz, CH_2CH_3); δ_C (125 MHz, DMSO-d_6) 172.6, 172.4, 172.3, 172.2 (4 x *C*ONH), 170.1 (*C*$ONHCH_2$), 52.3 ($NHCHCH_2$), 49.3, 48.5, 47.9 (3 x $NHCHCH_3$), 41.5 ($NHCH_2$), 35.5 (CH_2CO), 28.0 ($NHCHCH_2$), 27.8, 22.2 (2 x CH_2), 21.4 ($NHCH_2CH_2$), 18.9, 18.6, 18.5 (3 x $CHCH_3$), 14.2 (CH_2CH_3); m/z (ESI+) 412.3 ([M+H]+, 15.0%), 434.2 ([M+Na]+, 100.0%); HR-ESIMS: calculated for $C_{19}H_{33}N_5O_5Na$: 434.2374, found 434.2379 $[M+Na]^+$.

***N-tert*-Butoxycarbonyl-L-alanine benzyl ester 2.25**



Sodium hydride (60% dispersion in mineral oil, 0.32 g, 7.9 mmol) was washed with hexane and added with stirring to CH_2Cl_2 (25 mL) at 0 °C under nitrogen. Boc-L-Ala-OH (1.5 g, 7.9 mmol) was added, followed by benzyl bromide (1.63 g, 9.5 mmol). The reaction was stirred at 0 °C for 1 hour and at room temperature for 18 hours. TMEDA (5 mL) was added, the reaction mixture was partitioned between EtOAc (50 mL) and pH 2 buffer (100 mL), and the aqueous phase was extracted with EtOAc (2 x 50 mL). The organic extract was washed with pH 2 buffer (50mL), water (50 mL) and saturated aqueous $NaHCO_3$ (2 x 50 mL) dried over Na_2SO_4 and concentrated *in vacuo* to give **2.25** as a pale yellow oil (2.04 g, 7.3 mmol, 92 %); $[\alpha]_D^{28}$ -6.1 (c = 1.9, MeOH) (lit.[7] $[\alpha]_D^{25}$ -40.5 (c = 2.0, MeOH)); υ_{max}/cm^{-1} 3348 (N-H), 2978 (aromatic C-H), 2934 (aliphatic C-H), 1710 (ester C=O), 1696, (amide C=O); δ_H (400 MHz, $CDCl_3$) 7.39 - 7.28 (5H, m, 5 x Ar C*H*), 5.22 - 5.07 (3H, m, *CH*$_2$Ph, N*H*CH), 4.36 (1H, quin, *J* 7.5 Hz, N*H*CH), 1.43 (9H, s, C(C*H*$_3$)$_3$), 1.39 (3H, d, *J* 7.5 Hz, CHC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 173.0 (*C*O_2Bn), 155.1 (*C*$O_2{}^t$Bu), 135.7 (*ipso C*), 128.6, 128.3, 128.1 (*ortho*, *meta* and *para C*H), 79.7 (*C*$(CH_3)_3$), 66.8 (C*H*$_2$Ph), 49.3 (NHC*H*), 28.3 (C(C*H*$_3$)$_3$), 18.5 (CHC*H*$_3$). Data are consistent with that previously reported, with the exception of the optical rotation due to apparent racemisation during the reaction.[8]

(*N-tert*-butoxycarbonyl-L-alanyl)-L-alanine benzyl ester 2.26

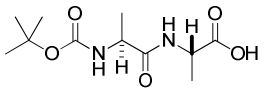


Acetyl chloride (3.1 mL, 44 mmol) was added dropwise to cooled and stirred MeOH (20 mL) at 0°C. The resulting solution was added to **2.25** (2.0 g, 7.3 mmol) and the reaction mixture stirred at room temperature for 18 hours. Complete deprotection was confirmed by TLC (1:1 EtOAc:hexane). The solvent was removed *in vacuo* to

give the crude L-alanine benzyl ester hydrochloride salt, which was used immediately.

A solution of the crude amine (7.3 mmol) and *N*-methyl morpholine (0.8 mL, 7.3 mmol) in EtOH (30 mL) was stirred at room temperature. Boc-L-Ala-OH (1.6 g, 8.0 mmol), Oxyma (0.16 g, 1.1 mmol) and *N*-methyl morpholine (1.7 mL, 16 mmol) were added and the reaction was cooled to 0 ˚C. EDCI (1.7 g, 8.7 mmol) was added and the reaction stirred overnight. The reaction mixture was concentrated *in vacuo* and the residue was partitioned between EtOAc (30 mL) and pH 2 buffer (30 mL). The separated organic phase was washed with pH 2 buffer (30 mL), water (30 mL) and saturated $NaHCO_3$ solution (30 mL) and saturated aqueous NaCl solution (30 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give **2.26** as a viscous orange oil (1.8 g, 5.14 mmol, 70 %) (;$[\alpha]_D^{25}$ -104.1 (c = 1.60, MeOH); (lit. [9] $[\alpha]_D^{22}$ = -60.2 (c = 1.0, MeOH)); υ_{max}/cm^{-1} 3321 (N-H), 2979 (Ar C-H), 2934 (aliphatic C-H), 1732 (ester C=O), 1682 (amide C=O), 1520 (N-H); δ_H (400 MHz, $CDCl_3$) 7.39 - 7.29 (5H, m, 5 x Ar C*H*), 6.80 (1H, br. s., CO_2NH), 5.20 - 5.10 (3H, m, CH_2Ph, CONHCH), 4.64 - 4.52 (1H, m, CO_2NHCH), 4.18 (1H, q, *J* 7.0 Hz, CONHC*H*), 1.43 (9H, s, $C(CH_3)_3$), 1.42 - 1.30 (6H, m, 2 x $CHCH_3$); δ_C (100 MHz, $CDCl_3$) 172.6, 172.3 (CO_2Bn, CONH), 155.5 ($^tBuCO_2NH$), 135.4 (*ipso C*), 128.6, 128.3, 128.1 (*ortho*, *meta* and *para* C*H*), 80.1 ($C(CH_3)_3$), 67.9 (CH_2Ph), 48.1, 48.0 (2 x NHCH), 28.3 ($C(CH_3)_3$), 18.4, 18.2 (2 x $CHCH_3$). Spectroscopic data are consistent with that previously reported.[10]

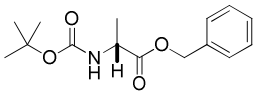
(*N-tert*-Butoxycarbonyl-L-alanyl)-L-alanine 2.27



10% Palladium on activated carbon (~20 mg) was added to a solution of **2.26** (1.2 g, 3.4 mmol) in MeOH (20 mL). The reaction mixture was purged with hydrogen and stirred at room temperature under 1 atm of hydrogen for 18 hours. The reaction was filtered over celite and concentrated *in vacuo* to give a white tacky waxy (0.58 g, 2.2

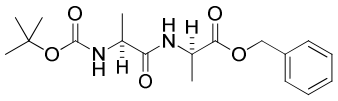
mmol, 65 %); $[\alpha]_D^{34}$ -20.4 (c = 1.0, MeOH), (lit.[11] $[\alpha]_D^{22}$ -39.1 (c = 1.0, MeOH)); υ_{max}/cm^{-1} 3321 (O-H), 2979 (aliphatic C-H), 1711 (ester C=O), 1660 (amide C=O); δ_H (400 MHz, $CDCl_3$) 7.34 - 7.24 (1H, br. s., CON*H*CH), 5.64 - 5.54 (1H, br. s., CO_2N*H*CH), 4.65 - 4.47 (1H, m, CONHC*H*), 4.33 (1H, br. s., CO_2NHC*H*), 1.54 - 1.33 (15H, m, C(C*H*$_3$)$_3$, 2 x CHC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 175.3, 173.0 (*C*ONH), 155.9 (^{t}Bu*C*O_2NH), 77.3 (*C*$(CH_3)_3$), 48.4, 48.0 (2 x NH*C*H), 28.3 (C(*C*H$_3$)$_3$), 18.7, 18.1 (2 x *C*H_3). Data are consistent with that previously reported.[9,12]

N-tert-Butoxycarbonyl-D-alanine benzyl ester 2.28



Method as for **2.25** gave the product as a yellow oil (2.1 g, 7.5 mmol, 95 %). $[\alpha]_D^{28}$ +37.6 (c = 1.52, MeOH) (lit. [13] $[\alpha]_D^{25}$ +23.2 (c = 1.5, MeOH)); υ_{max}/cm^{-1} 3332 (N-H), 2977 (aromatic C-H), 2934 (aliphatic C-H), 1710 (ester C=O), 1669 (amide C=O); δ_H (400 MHz, $CDCl_3$) 7.37 - 7.16 (5H, m, 5 x Ar C*H*), 5.22 - 5.00 (3H, m, C*H*$_2$Ph, NHC*H*), 4.27 (1H, quin, *J* 7.5 Hz, NHC*H*), 1.35 (9H, s, C(C*H*$_3$)$_3$), 1.30 (3H, d, *J* 7.5 Hz, CHC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 173.2 (*C*O_2Bn), 155.2 (^{t}Bu*C*O_2NH), 135.5 (*ipso C*), 128.6, 128.3, 128.1 (*ortho*, *meta* and *para* CH), 79.7 (*C*$(CH_3)_3$), 66.9 (*C*H_2Ph), 49.3 (NH*C*H), 28.3 (C(*C*H$_3$)$_3$), 18.5 (CH*C*H$_3$); m/z (ESI+) 302.1 ([M+Na]+, 100%), 580.7 ([[2 x M]+Na], 7.7%); HR-ESIMS: calculated for $C_{13}H_{16}NO_4Na$: 302.1363, found 302.1369 $[M+Na]^+$. Spectroscopic data are consistent with that previously reported.[14]

(_N-tert_-Butoxycarbonyl-L-alanyl)-D-alanine benzyl ester 2.29

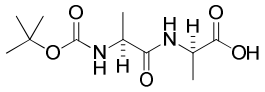


Acetyl chloride (3.4 mL, 48 mmol) was added dropwise to cooled and stirred MeOH (20 mL) at 0°C. The resulting solution was added to **2.28** (2.2 g, 7.9 mmol) and the reaction mixture stirred at room temperature for 18 hours. Complete deprotection

was confirmed by TLC (1:1 EtOAc:hexane). The solvent was removed *in vacuo* to give the crude D-alanine benzyl ester hydrochloride salt, which was used immediately.

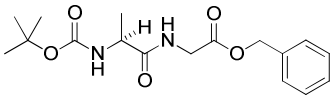
A solution of the crude amine (7.9 mmol) and *N*-methyl morpholine (0.9 mL, 7.9 mmol) in EtOH (30 mL) was stirred at room temperature. Boc-L-Ala-OH (1.7 g, 8.7 mmol), Oxyma (0.17 g, 1.2 mmol) and *N*-methyl morpholine (1.9 mL, 17 mmol) were added and the reaction was cooled to 0 ˚C. EDCI (1.8 g, 9.7 mmol) was added and the reaction stirred overnight. The reaction mixture was concentrated *in vacuo* and the residue was partitioned between EtOAc (30 mL) and pH 2 buffer (30 mL). The separated organic phase was washed with pH 2 buffer (30 mL), water (30 mL) and saturated $NaHCO_3$ solution (30 mL) and saturated aqueous NaCl solution (30 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give **2.29** as a viscous orange oil afforded the coupled product as a waxy low melting point orange solid (2.14 g, 6.7 mmol, 81 %); $[\alpha]_D^{25}$ -15.2 (c = 2.20, MeOH); υ_{max}/cm^{-1} 3332 (N-H), 2978 (aromatic C-H), 2931 (aliphatic C-H), 1666 (amide C=O), 1518 (N-H); δ_H (400 MHz, $CDCl_3$) 7.37 - 7.29 (5H, m, Ar C*H*), 6.93 - 6.85 (1H, br. s., $^tBuCO_2NH$), 5.20 - 5.09 (3H, m, CH_2Ph, CON*H*), 4.64 - 4.53 (1H, m, $^tBuCO_2NHCH$), 4.29 - 4.14 (1H, m, CONHC*H*), 1.46 - 1.41 (9H, m, $C(CH_3)_3$), 1.41 - 1.36 (6 H, m, 2 x $CHCH_3$); δ_C (100 MHz, $CDCl_3$) 172.6, 172.4 (CO_2Bn, *C*ONH), 155.5 ($^tBuCO_2NH$), 135.4 (*ipso C*), 128.6, 128.3, 128.1 (*ortho, meta* and *para* CH), 80.1 ($C(CH_3)_3$), 67.1 (CH_2Ph), 48.1, 48.0 (2 x $CHCH_3$), 28.3 ($C(CH_3)_3$), 18.6, 18.2 (2 x $CHCH_3$); m/z (ESI+) 373.1 ([M+Na]+, 100%); HR-ESIMS: calculated for $C_{18}H_{26}N_2O_5Na$: 373.1734, found 373.1740 $[M+Na]^+$. This compound is known but no data has been previously reported.[15]

(*N-tert*-Butylcarbonyl-L-alanyl)-D-alanine 2.30



10% Palladium (~20 mg) on activated carbon was added to a solution of **2.29** (1.2 g, 3.4 mmol) in MeOH (20 mL). The reaction mixture was purged with hydrogen and stirred at room temperature under a constant positive pressure of hydrogen for 18 hours. The reaction was filtered over celite and concentrated *in vacuo* to give **2.30** as a colourless oil (0.86 g, 3.3 mmol, 97 %); $[\alpha]_D^{25}$ -34.1 (c = 0.13, MeOH); υ_{max}/cm^{-1} 3317 (O-H), 2979 (aliphatic C-H), 1712 (acid C=O), 1660 (amide C=O); δ_H (300 MHz, $CDCl_3$) 8.74 (1H, br. s., CO_2H), 7.23 (1H, d, *J* 7.0, CONHCH), 5.75 - 5.48 (1H, m., CO_2NHCH, major rotamer), 5.42 - 5.00 (1H, m., CO_2NHCH, minor rotamer), 4.54 - 4.31 (1H, m, CONHCH), 4.33 - 4.14 (1H, m, CO_2NHCH), 1.58 - 1.03 (15H, m, $C(CH_3)_3$, 2 x $CHCH_3$); δ_C (100 MHz, $CDCl_3$) 175.4, 172.8 (CONH, CO_2H), 155.8 ($^t BuCO_2$), 77.4 ($C(CH_3)_3$), 48.2, 48.1 (2 x NHCH), 28.3 ($C(CH_3)_3$), 18.7, 18.2 (2 x $CHCH_3$). This compound is known but only the ^{15}N NMR spectrum has been reported.[16]

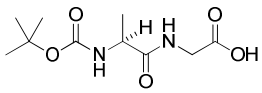
(*N-tert*-Butoxycarbonyl-L-alanyl)glycine benzyl ester 2.31



Method modified from literature procedure of Pu *et al.*[17] *N*-Methyl morpholine (1.72 ml, 15.8 mmol) was added to a solution of glycine benzyl ester *p*-toluenesulfonate (2.81 g, 8.3 mmol) in EtOH (10 mL) to give a white suspension. Te mixture was cooled to 0 - 5°C, and Oxyma (0.17 g, 1.2 mmol) was added, followed by Boc-L-alanine (1.50 g, 7.9 mmol) and *N*-methyl morpholine (1.0 mL, 9.6 mmol). EDCI (1.82 g, 9.5 mmol) was added and the reaction was stirred to room temperature over 16 hours to give a dark orange solution. The reaction mixture was concentrated *in vacuo* and the residue was partitioned between CH_2Cl_2 (30 mL) and pH 2 buffer (30 mL). The separated organic phase was washed with pH 2 buffer (30 mL), water

(30mL) and saturated NaHCO3 solution (30mL) and saturated aqueous NaCl solution (30 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give a bright orange oil. This was purified by silica chromatography (75 % EtOAc: hexanes) to give **2.31** as a bright white solid (2.12 g, 6.30 mmol, 80 %, Rf = 0.29); m.p. 88 - 89 °C, (lit.[18] 83 - 84 °C); $[\alpha]_D^{33}$ -23.4 (c = 1.20, MeOH), (lit.[6] $[\alpha]_D^{20}$ -25.4 (c = 1.26, MeOH)); υ_{max}/cm^{-1} 3322 (N-H), 2972 (aromatic C-H), 2934 (aliphatic C-H), 1734 (ester C=O), 1682, 1653 (amide C=O), 1523 (N-H); δ_H (400 MHz, DMSO-d_6) 8.22 (1H, t, *J* 6.0 Hz, N*H*CH_2), 7.50 - 7.23 (5H, m, 5 x Ar C*H*), 6.92 (1H, d, *J* 7.5 Hz, NHCH), 5.12 (2H, s, CH_2Ph), 4.07 - 3.90 (3H, m, NHC*H*, NHCH_2), 1.37 (9H, s, C(CH_3)$_3$), 1.17 (3H, d, *J* 7.5 Hz, CHCH_3); δ_C (100 MHz, DMSO-d6) 173.3 (*C*ONH), 169.7 (*C*O_2Bn), 155.0 (*C*O_2^{t}Bu), 135.9 (*ipso C*), 128.4, 128.0, 127.9 (*ortho*, *meta* and *para* CH), 78.0 (*C*$(CH_3)_3$), 65.8 (*C*H_2Ph), 49.5 (NH*C*H), 40.7 (NH*C*H_2), 28.2 (C(*C*H_3)$_3$), 18.2 (CH*C*H_3); m/z (ESI+) 359.1 ([M+Na]+, 100%); HR-ESIMS: calculated for $C_{17}H_{24}N_4O_5Na$: 359.1577, found 359.1578 $[M+Na]^+$.Data are consistent with that previously reported.[19]

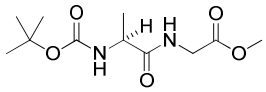
(*N-tert*-butoxycarbonyl-L-alanyl)glycine 2.32



(*N-tert*-butoxycarbonyl-L-alanyl)glycine benzyl ester (1.5 g, 4.46 mmol) was dissolved in MeOH (15 mL) and palladium (10% on activated carbon, ~20 mg) was added. The mixture was purged with hydrogen and stirred at 1 atm of hydrogen atmosphere for 12 hours. The reaction mixture was filtered over celite and the filtrate concentrated *in vacuo* to afford **2.32** as a clear oil (1.2 g, 4.45 mmol, 99 %); $[\alpha]_D^{28}$ -22.1 (c = 0.95, MeOH), (lit.[20] $[\alpha]_D^{20}$ -26.9 (c = 0.98, MeOH)); δ_H (400 MHz, DMSO-d_6) 8.00 (1H, t, *J* 6.0 Hz, N*H*CH_2), 6.89 (1H, d, *J* 8.0 Hz, N*H*CH), 4.01 (1H, quin, *J* 7.5 Hz, NHC*H*), 3.79 (1H, dd, *J* 17.5, 6.0 Hz, NHCH_2), 3.71 (1H, dd, *J* 17.5, 6.0 Hz, NHCH_2), 1.38 (9H, s, C(CH_3)$_3$), 1.19 (3H, d, *J* 7.0 Hz, CHCH_3); δ_C (100 MHz, DMSO-d_6) 173.0 (*C*ONH), 171.1 (*C*O_2H), 155.0 (*C*O_2^{t}Bu), 78.0 (*C*$(CH_3)_3$), 49.5

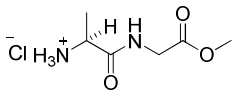
(NH*C*H), 40.7 (NH*C*H_2), 28.1 (C(*C*H_3)$_3$), 18.1 (CH*C*H_3); m/z (ESI+) 161.1 ([M+Na]+, 100%); HR-ESIMS: calculated for $C_{10}H_{18}N_2O_5Na$: 269.1108, found 269.1111 $[M+Na]^+$. Data are consistent with that previously reported.[21]

(*N-tert*-butoxycarbonyl-L-alanyl)glycine methyl ester 2.33



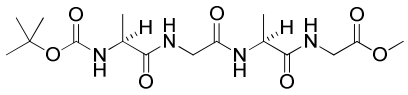
Method modified from literature procedure of Pu *et al*.[17] *N*-Methyl morpholine (1.7 ml, 15.0 mmol) was added to a solution of glycine methyl ester hydrochloride (1.98 g, 15.8 mmol) in EtOH (15 mL) to give a white suspension. The mixture was cooled to 0-5°C, and Oxyma (0.32 g, 2.3 mmol) was added, followed by Boc-L-alanine (2.84 g, 15.0 mmol) and *N*-methyl morpholine (3.5 mL, 32.2 mmol). EDCI (5.75 g, 30.0 mmol) was added and the reaction was stirred to room temperature over 16 hours to give a dark orange solution. The reaction mixture was concentrated *in vacuo* and the residue was partitioned between CH_2Cl_2 (30 mL) and pH 2 buffer (30 mL). The separated organic phase was washed with pH 2 buffer (30 mL), water (30mL) and saturated $NaHCO_3$ solution (30mL) and saturated aqueous NaCl solution (30 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give a bright orange oil. This was purified by silica chromatography (EtOAc) to give **2.33** as a clear oil (3.3 g, 12.5 mmol, 83 %, Rf = 0.15); $[\alpha]_D^{28}$ -27.0 (c = 1.04, MeOH), (lit. $[\alpha]_D^{20}$ -28.4 (c = 1.0, MeOH)); υ_{max}/cm^{-1} 3307 (N-H), 2978 (aliphatic C-H), 1741 (ester C=O), 1659 (amide C=O); δ_H (400 MHz, DMSO-d_6) 8.18 (1H, t, *J* 6.0 Hz, N*H*CH_2), 6.93 (1H, d, *J* 7.5 Hz, N*H*CH), 4.03 (1H, quin., *J* 7.0 Hz, NHC*H*), 3.88 (1H, dd, *J* 17.5, 6.5 Hz, NHCH_2), 3.81 (1H, dd, *J* 17.5, 6.5 Hz, NHCH_2), 3.64 (s, 3H, OCH_3), 1.39 (9H, s, C(CH_3)$_3$), 1.20 (3H, d, *J* 7.0 Hz, CHCH_3); δ_C (100 MHz, DMSO-d_6) 173.0 (*C*ONH), 169.6 (*C*O_2Me), 155.0 (*C*$O_2{}^t$Bu), 78.0 (*C*$(CH_3)_3$), 50.8 (O*C*H_3), 48.5 (NH*C*H), 40.7 (NH*C*H_2), 28.1 (C(*C*H_3)$_3$), 18.1 (CH*C*H_3); m/z (ESI+) 283.1 ([M+Na]+, 100%); HR-ESIMS: calculated for $C_{11}H_{20}N_2O_5Na$: 283.1264, found 283.1260 $[M+Na]^+$. Data are consistent with that previously reported.[22]

L-Alanylglycine methyl ester hydrochloride 2.34



2.33 (2.7 g, 10.4 mmol) was stirred in methanolic hydrochloric acid solution (2M, 40 mL) for 9 hours. The reaction was deemed complete by TLC (50% EtOAc:Hexane) and concentrated *in vacuo* to afford the product as a white sticky solid (1.7 g, 8.64 mmol, 83 %); $[\alpha]_D^{34}$ +10.2 (c = 0.59, MeOH), (lit.[23] $[\alpha]_D^{27.5}$ +11.6 (c = 2.41, DMF)); υ_{max}/cm^{-1} 2939 (br., N-H), 1736 (ester C=O), 1672 (amide C=O), 1555 (N-H); δ_H (400 MHz, DMSO-d_6) 9.12 - 9.01 (1H, m, N*H*CH), 8.39 (3H, br. s., N*H*$_3$CH), 4.01 - 3.82 (3H, m, NH_3CH, $NHCH_2$), 3.65 (3H, s, OCH_3), 1.46 - 1.35 (3H, m, $CHCH_3$); δ_C (100 MHz, DMSO-d_6) 170.9 (*C*ONH), 169.3 (CO_2Me), 51.8 (OCH_3), 47.7 (NH*C*H), 40.6 ($NHCH_2$), 17.1 ($CHCH_3$); m/z (ESI+) 161.1 ([M-Cl]+, 77.5%), 139.2 ([M-$ClNH_3CHCH_3$, +Na]+, 22.5%); HR-ESIMS: calculated for $C_6H_{13}N_2O_3$: 161.0921, found 161.0922 $[M+-Cl]^+$. ^{1}H NMR data are consistent with that previously reported.[24]

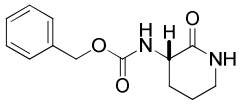
(*N-tert*-butoxycarbonyl-L-alanyl)glycyl-L-alanylglycine methyl ester 2.35



Method modified from literature procedure of Pu *et al.*[17] To a solution of **2.34** (4.90 g, 19.9 mmol) and N-methyl morpholine (2.16 mL, 19.9 mmol) in EtOH (20 mL) was added Oxyma (0.42 g, 3.00 mmol), **2.32** (4.33 g, 22.0 mmol) and *N*-methyl morpholine (4.76 mL, 43.8 mmol), and the resulting yellow suspension cooled to 0 °C on ice. After stirring for 10 minutes, EDCI (4.60 g, 23.9 mmol) was added and the reaction stirred to room temperature overnight. The resultant orange solution was concentrated *in vacuo* and the crude product isolated by silica chromatography (10-15 % MeOH:$CHCl_3$). Repeated trituration (EtOAc/hexanes) afforded the product as a cream powdery solid (0.99 g, 2.54 mmol, 13 %); m.p. 192 - 193 °C; $[\alpha]_D^{33}$ -25.4 (c = 1.0, MeOH); υ_{max}/cm^{-1} 3307, 3278 (N-H), 2978 (aliphatic C-H), 1721 (ester C=O),

1671, 1633 (amide C=O); δ_H (400 MHz, $CDCl_3$) 7.48 (1H, t, *J* 5.0 Hz, N*H*CH_2), 7.44 - 7.36 (2H, m, N*H*CH_2, N*H*CH), 5.50 (1H, d, *J* 5.5 Hz, $^tBuCO_2N$*H*), 4.67 (1H, quin., *J* 7.5 Hz, NHC*H*), 4.33 - 4.23 (1H, m, $^tBuCO_2NHC$*H*), 4.11 - 4.00 (4H, m, 2 x NHCH_2), 3.76 (3H, s, OCH_3), 1.47 - 1.43 (12H, m, (C(CH_3)$_i$, CHCH_3), 1.41 (3H, d, *J* 7.5 Hz, CHCH_3); δ_C (100 MHz, $CDCl_3$) 173.9, 172.9, 170.3 (3 x *C*ONH), 169.0 (*C*O_2CH_3), 155.9 (*C*$O_2{}^t$Bu), 80.4 (*C*$(CH_3)_3$), 52.3 (O*C*H_3), 50.6, 49.1 (2 x NH*C*H), 43.3, 41.1 (2 x *C*H_2), 28.3 (C(*C*H_3)$_3$), 18.5, 18.1 (2 x CH*C*H_3); m/z (ESI+) 411.1 ([M+Na]+, 100%); HR-ESIMS: calculated for $C_{16}H_{28}N_4O_7Na$: 411.1850, found 411.1851 $[M+Na]^+$.

(*S*)-3-Benzyloxycarbonylaminopiperidine-2-one 2.37

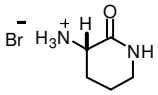


L-Ornithine hydrochloride (10.0 g, 59.3 mmol) was added to a stirred solution of acetyl chloride (9.3 mL, 118.6 mmol) in MeOH (50 mL) at 0 ºC. The mixture was heated to reflux for 3 hours, after which the reaction was seen to be complete by ^{1}H NMR affording the crude methyl ester (59.3 mmol, assuming complete conversion) in solution, which was used immediately without further purification.

The lactam was synthesized using a method modified from the literature procedure of Abe *et al*.[25] The crude methyl ester (59.3 mmol) was cooled to 0 ºC under N_2 with stirring and sodium methoxide solution in MeOH (25% w/w, 128.2 mmol, 28.0 mL) was added over 2 hours at 5-10 ºC. The reaction mixture was stirred for 2 hours at 0 ºC and then was concentrated *in vacuo* at 20 ºC to give the crude lactam as a waxy white solid. This was dissolved in THF (50 mL), cooled to 0 °C, and water (50mL) and sodium bicarbonate (9.96 g, 118.6 mmol) were added. Benzyl chloroformate (12.5 mL, 83.1 mmol) was added slowly, maintaining the reaction temperature below 5ºC. The reaction was held at below 5 ºC for 3 hours, then allowed to stir at room temperature over 72 hours to afford a biphasic mixture. To this was added EtOAc (50 mL) and water (50 mL) and the phases were separated. The aqueous

phase was further extracted with EtOAc (2 x 30 mL) and the combined organics were washed with aqueous HCl solution (1M, 50 mL), aqueous saturated Na_2CO_3 solution (50 mL) and saturated aqueous NaCl solution (50 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the crude product as a yellow oil. Recrystallisation from EtOAc and hexanes gave **2.37** as a glassy, white crystalline solid (12.4 g, 50.0 mmol, 84 %); m.p. 114 - 116 ºC (lit. [25]101 - 102 °C) ; $[\alpha]_D^{26}$+35.2 (c=1.0, $CHCl_3$), $[\alpha]_D^{34}$-11.9 (c=1.0, MeOH), (lit.[25] $[\alpha]_D^{23}$-18.5 (c=1.0, MeOH)); υ_{max}/cm^{-1} 3324 (N-H), 1683 (carbamate C=O), 1655, 1617 (lactam C=O), 1521 (C=C); δ_H (400 MHz, $CDCl_3$) 7.57 - 7.02 (5 H, m, 5 x Ar CH), 6.46 (1H, s, $CONHCH_2$), 5.85 (1H, d, J 4.5 Hz, CHNH), 5.14 (2H, m, $PhCH_2$), 4.11 (1H, dt, J 11.5, 5.5 Hz, $NHCHCH_2$), 3.31 (2H, t, J 5.0 Hz, $NHCH_2$), 2.64 - 2.36 (1H, m, $NHCHCH_2$), 2.00 - 1.79 (2H, m, $NHCH_2CH_2$), 1.64 (1H, dtd, J 13.0, 11.5, 5.5 Hz, $NHCHCH_2$); δ_C (100 MHz, $CDCl_3$) 171.5 (CHCONH), 156.4 ($NHCO_2$), 136.5 (ipso C), 128.5, 128.1, 127.0 (ortho, meta, para CH), 66.8 ($PhCH_2$), 51.7 ($NHCHCH_2$), 41.7($NHCH_2$), 27.4, 21.0 (2 x CH_2); m/z (ESI+) 271.1 ([M+Na]+, 100%); HR-ESIMS: calculated for $C_{13}H_{16}N_2O_3Na$: 271.1052, found 271.1063 $[M+Na]^+$. Enantiomeric excess: >99.9% by HPLC by comparison with known standards. The data are consistent with that previously reported.[26]

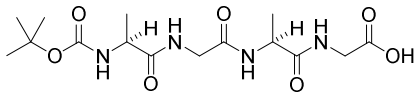
_S_-(-)-3-aminopiperidine-2-one hydrobromide salt 2.38



The amine was deprotected using a method modified from literature procedure of Ben-Ishai *et al.*[27] **2.37** (1.0 g, 4.03 mmol) was stirred ina solution hydrobromic acid in acetic acid (45 % w/w solution, 4.0mL, 22.0 mmol) for 2 hours. The resulting solid was washed with ether (2 x 30 mL) and dried by toluene azeotrope to give **2.38** as a beige solid (0.78 g, 4.01 mmol, 99 %); m.p. 179 - 180ºC; $[\alpha]_D^{26}$+10.2 (c=1.0, MeOH); υ_{max}/cm^{-1} 2916 (N-H), 2392 (aliphatic C-H), 1737 (lactam C=O), 1654, 1578 (amide C=O); δ_H (400 MHz, D_2O) 3.45 - 3.22 (1H, m, NH_3CH), 3.26 - 3.20

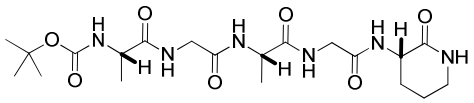
(2H, m, NHCH_2), 2.37 - 2.18 (1H, m, NHCHCH_2), 2.14 - 1.67 (3H, m, NHCH$_2$CH_2, NHCHCH_2); δ_C (100 MHz, D_2O) 170.1 (*C*ONH), 52.3 (*C*HNH$_3$), 41.3 (NH*C*H$_2$), 24.6, 19.3 (2 x *C*H$_2$); m/z (ESI+) 115.3 ([M-Br]+, 70.6%), 137.2 ([M+Na]+, 22%); HR-ESIMS: calculated for $C_5H_{11}N_2O$: 115.0866, found 115.0865 $[M-Br]^+$. Data are consistent to that previously reported. [25]

(*N-tert*-butoxycarbonyl-L-alanyl)glycyl-L-alanylglycine 2.36



To a solution of **2.35** (0.99 g, 2.54 mmol) in EtOH (8 mL) was added a solution of lithium hydroxide (0.12 g, 5.09 mmol) in EtOH and water (1:1, 2 mL). The resulting suspension was stirred at room temperature for 8 hours. A sample was concentrated *in vacuo* at <30 °C, and complete hydrolysis of the ester confirmed by ^{1}H NMR. The bulk product was not isolated, and was used as the solution in EtOH.

(3*S*)-3-[(*N-tert*-Butoxycarbonyl-L-alanyl)glycyl-L-alanylglycyl]-aminopiperidine-2-one 2.39



N-Methylmorpholine (0.67 mL, 6.18 mmol) was added to a solution of S-(-)-3-aminopiperidine-2-one hydrobromide salt (1.21 g, 6.18 mmol) in EtOH (5 mL). After stirring for 5 minutes, the resulting white suspension was added to an ethanolic solution of **2.36** (10 mL, 2.54 mmol), Oxyma (0.054 g, 0.38 mmol) and *N*-methylmorpholine (0.32 mL, 3.05 mmol). The reaction mixture was cooled to 0 °C on ice and stirred for 10 minutes, after which EDCI (0.56 g, 3.06 mmol) was added and the reaction was stirred at room temperature over 16 hours. The resulting orange solution was concentrated *in vacuo* and the crude product isolated by silica chromatography (15 - 20 % MeOH:$CHCl_3$), along with trace amounts of *N*-methylmorpholine hydrochloride and probable Oxyma hydrolysis products. **2.39** was

obtained by reverse phase HPLC and lyophilisation as white shiny platelets (0.14 g, 0.30 mmol, 12%); m.p. 122 - 124 °C; $[\alpha]_D^{26}$ -2.31 (c = 0.23, MeOH); υ_{max}/cm^{-1} 3286 (br., N-H), 3074 (aromatic C-H), 2981 (aliphatic C-H), 1649 (amide C=O), 1529 (N-H); δ_H (700 MHz, CD_3OD) 4.26 - 4.22 (1H, m, NHC*H*CH_2), 4.22 - 4.18 (1H, m, NHC*H*CH_3), 3.97 - 3.94 (1H, m, NHC*H*CH_3), 3.90 - 3.70 (4H, m, 2 x NHC*H*$_2$CO), 3.32 - 3.27 (2H, m, NHC*H*$_2$$CH_2$), 2.02 - 1.95 (1H, m, NHCHC*H*$_2$), 1.86 - 1.81 (1H, m, $NHCH_2$C*H*$_2$), 1.80 - 1.66 (2H, m, NHCHC*H*$_2$, $NHCH_2$C*H*$_2$), 1.35 (9H, s, C(C*H*$_3$)$_3$), 1.32 - 1.29 (3H, m, CHC*H*$_3$), 1.26 - 1.20 (3H, m, CHC*H*$_3$); δ_C (175 MHz, CD_3OD) 175.5, 173.9, 171.4, 170.4, 170.0 (5 x *C*ONH), 156.8 (*C*O_2^{t}Bu), 79.5 (*C*$(CH_3)_3$), 50.6 (^{t}BuCO$_2$NH*C*H), 49.6, 49.4 (NH*C*HCH_2, NH*C*HCH_3), 42.6, 42.3 (2 x NH*C*H_2CO), 42.2 (NH*C*$H_2$$CH_2$), 27.2 (C(*C*$H_3$)$_3$), 27.0 (NHCH*C*$H_2$), 21.0 ($NHCH_2$*C*$H_2$), 16.5, 16.0 (2 x CH*C*$H_3$); m/z (ESI+) 493.1 ([M+Na]+, 100%), 244.2 ([M-$C_5H_9O_2$]+, 50%); HR-ESIMS: calculated for $C_{20}H_{35}N_6O_7$: 471.2562, found 471.2560 $[M+H]^+$.

2.6 EXPERIMENTAL REFERENCES

1. A. Rosowsky, C. M. Vaidya, H. Bader, J. E. Wright and B. A. Teicher, *J. Med. Chem.*, 1997, **40**, 286-299.
2. D. R. Clarke and E. S. Waight, *J. Chem. Soc. C*, 1971, 3743-3748.
3. M. D. Lloyd, K. D. Merritt, V. Lee, T. J. Sewell, B. Wha-Son, J. E. Baldwin, C. J. Schofield, S. W. Elson, K. H. Baggaley and N. H. Nicholson, *Tetrahedron*, 1999, **55**, 10201-10220.
4. N. A. e. a. Poddubnaya, *J. Gen. Chem. USSR (Engl. Transl.)*, 1966, **36**, 219-224, 228-232.
5. V. Burichenko, R. Kamilova and V. Shibnev, *Chem. Nat. Compd.*, 1974, **10**, 207-211.
6. A. Koohang, J. L. Bailey, R. M. Coates, H. K. Erickson, D. Owen and C. D. Poulter, *J. Org. Chem.*, 2010, **75**, 4769-4777.

7. S.-T. Chen and K. T. Wang, *J. Chem. Soc., Chem. Commun.*, 1988, 327-328.

8. K. C. Nadimpally, K. Thalluri, N. B. Palakurthy, A. Saha and B. Mandal, *Tetrahedron Lett.*, 2011, **52**, 2579-2582.

9. M. Narita, S.-i. Itsuno, M. Hirata and K. Kusano, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 1028-1033.

10. Y. Hamuro and A. D. Hamilton, *Bioorg. Med. Chem.*, 2001, **9**, 2355-2363.

11. M. Akiyama, K. Shimizu and M. Narita, *Tetrahedron Lett.*, 1976, **17**, 1015-1018.

12. R. J. Anderson, P. W. Groundwater, Y. Huang, A. L. James, S. Orenga, A. Rigby, C. Roger-Dalbert and J. D. Perry, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 832-835.

13. B. Xu, S.-F. Zhu, X.-L. Xie, J.-J. Shen and Q.-L. Zhou, *Angew. Chem., Int. Ed.*, 2011, **50**, 11483-11486.

14. C. Henneuse, T. Boxus, L. Tesolin, G. Pantano and J. Marchand-Brynaert, *Synthesis*, 1996, **1996**, 495-501.

15. T. P. Pathak and S. J. Miller, *J. Am. Chem. Soc.*, 2012, **134**, 6120-6123.

16. H. R. Kricheldorf and W. E. Hull, *Org. Magn. Reson.*, 1979, **12**, 607-611.

17. Y. J. Pu, R. K. Vaid, S. K. Boini, R. W. Towsley, C. W. Doecke and D. Mitchell, *Org. Process Res. Dev.*, 2008, **13**, 310-314.

18. R. D. Cowell and J. H. Jones, *J. Chem. Soc., Perkin Trans. 1*, 1972, 1809-1814.

19. V. Strukil, B. Bartolec, T. Portada, I. Dilovic, I. Halasz and D. Margetic, *Chem. Commun. (Cambridge, U. K.)*, 2012, **48**, 12100-12102.

20. H. Moser, A. Fliri, A. Steiger, G. Costello, J. Schreiber and A. Eschenmoser, *Helv. Chim. Acta*, 1986, **69**, 1224-1262.

21. S. K. Maji, D. Haldar, M. G. B. Drew, A. Banerjee, A. K. Das and A. Banerjee, *Tetrahedron*, 2004, **60**, 3251-3259.

22. G. Naturale, M. Lamblin, C. Commandeur, F.-X. Felpin and J. Dessolin, *Eur. J. Org. Chem.*, 2012, **2012**, 5774-5788.

23. B. J. Johnson and E. G. Trask, *J. Chem. Soc. C*, 1969, 2644-2647.

24. G. G. Smith and R. Baum, *J. Org. Chem.*, 1987, **52**, 2248-2255.

25. M. Abe, T. Akiyama, H. Nakamura, F. Kojima, S. Harada and Y. Muraoka, *J. Nat. Prod.*, 2004, **67**, 999-1004.

26. E. R. Alexander and E. J. Underhill, *J. Am. Chem. Soc.*, 1949, **71**, 4014-4019.

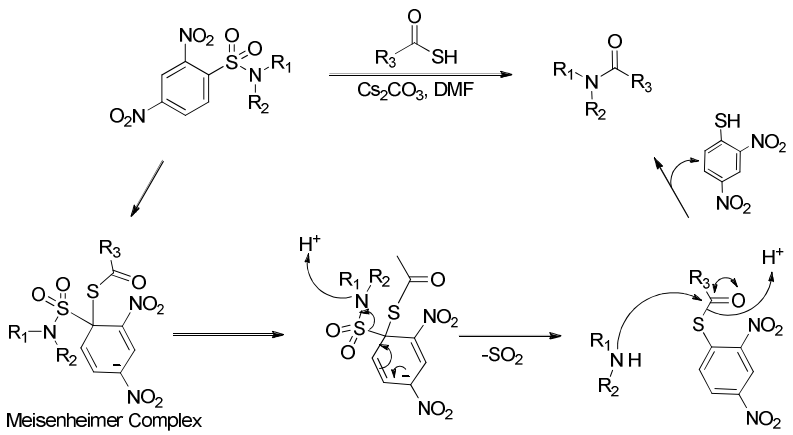
27. D. Ben-Ishai and A. Berger, *J. Org. Chem.*, 1952, **17**, 1564-1570.

CHAPTER 3: THIOACIDS AND ISOLEUCINE

3.1 THIOACIDS FOR PEPTIDE COUPLING

Thioacid mediated amide bond formation is seen as a particularly attractive alternative to standard peptide couplings, as they react quite differently from carboxylic acids, allowing amide bond formation by direct reaction with azides,[1, 2] isonitriles,[3] isocyanates[4] and sulfonamides[5-8] without the need for traditional peptide coupling reagents.[9]

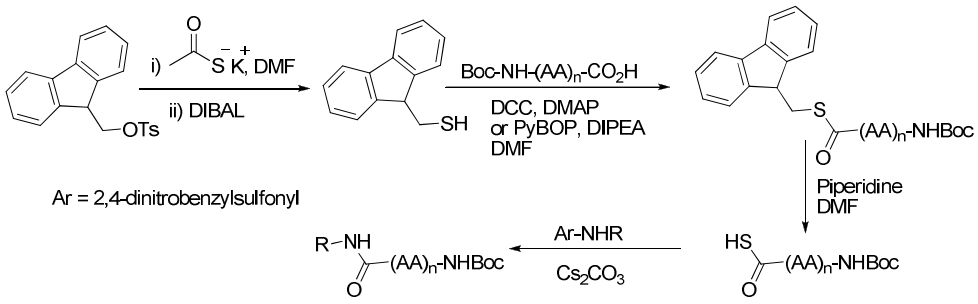
In 1998, Tomkinson *et al.* described the treatment of a series of 2,4-nitrobenzenesulfonamides with thioacids in the presence of cesium carbonate to provide the corresponding mono- and di-substituted amide (Scheme 6).[7, 8] These reactions proceed *via* nucleophilic substitution by the thioacid on the extremely electron poor sulfonamide to give the intermediate Meisenheimer complex. The collapse of this leads to loss of sulfur dioxide and the formation of the extremely reactive thioester, with introduction of the amine leading to the rapid formation of the amide bond.



Scheme 6: The route to amide bond formation using an aromatic sulfonamide and thioacid

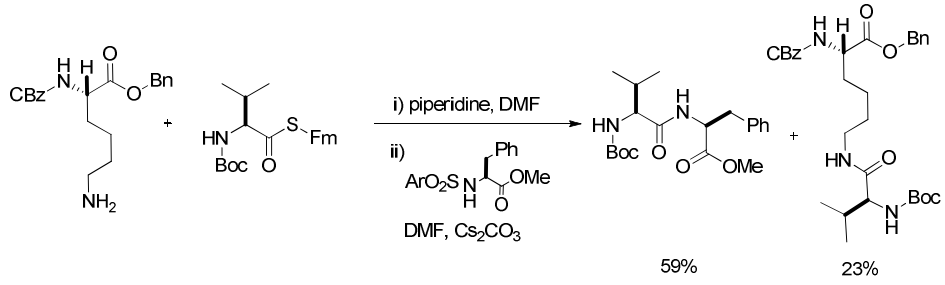
The original paper only studied the reaction of the achiral sulfonamides with achiral thioacids. In addition, the scope for the reaction seemed to be limited by the lack of commercially available thioacids, while most laboratory preparations of thioacids

required H_2S, which is both toxic and extremely malodorous.[10, 11] It was not until 2007, when Crich *et al.* revisited the chemistry, that the synthesis of a peptidyl thioacid was linked with the synthesis of a peptidyl sulfonamide to allow a block synthesis of a peptide.[5] Cleavage of a preformed fluorenylmethyl thioester provided a convenient source of a thioacid for further reaction (Scheme 7). This chemistry was successfully applied to the synthesis of a number of peptides, from small dipeptides to larger linear and cyclic hexapeptides.[6, 12, 13 14]



Scheme 7: Amide formation using the Crich method of thioacid preparation, *via* a fluorenomethylthiol donor

Importantly, the reaction appeared to proceed with very low levels of epimerization, reportedly lower than a variety of commonly used peptide coupling agents including PyBOP, HATU, HOBt and ECDI.[12] Further investigation showed that, though the thioacid reacted preferentially with the sulfonamide, unprotected amines would also react with the thioacid to form amides (Scheme 8).

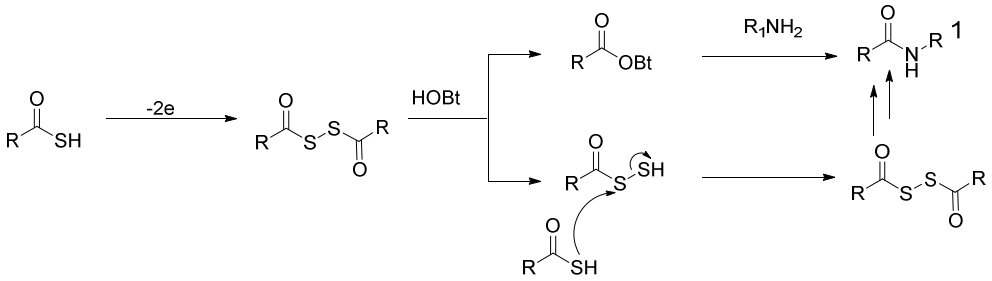


Scheme 8: Reaction of thioacids with free amines compared to sulphonamides

Wang *et al.* noticed a similar result when attempting to form amides in a three component reaction using isocyanates, thioacids and amines.[15] The planned reaction involved the initial formation of a thioformimidate carboxylate (thio-FMCA) mixed anhydride, which could then be intercepted by an amine to form the amide bond. In

order to prevent the rapid conversion of the thio-FMCA to the corresponding oxazolone, the reaction was performed in the presence of HOBt. However, control reactions which excluded the isocyanate showed comparable yields of the corresponding peptides.

When an oxidising agent such as iodine was added, conversions of up to 89% were observed. It is proposed that oxidative initiation is involved, possibly by the formation of a disulphide.[16] This activated species can then react with HOBt to form both the HOBt ester and the reactive perthio- species, from which the disulphide can be reformed. (Scheme 9).



Scheme 9: Proposed oxidative initiation reaction to form amides[15]

It appeared that, due to the unique properties of thioacids, coupling agents are not necessarily required for rapid, epimerisation-free, amide bond formation. A literature search provided multiple earlier examples of the oxidative coupling of thioacids to produce amides.[17-20]

It should be noted that block-peptide coupling reactions between thioesters and a variety of peptide fragments have also been reported.[21] The thioesters are prepared using similar conditions to Crich *et al.* (Scheme 7), but are then reacted directly with the relevant amine without being deprotected to the relevant thioacid, and without the need for additional coupling reagents.

3.1.1 THIOACIDS FOR BLOCK PEPTIDE SYNTHESIS

The use of the Crich method of thioacid synthesis, followed by amide formation seemed to provide a promising alternative route to the block synthesis of small molecule CTLPs. Ultimately, we hoped to investigate whether a peptide coupling reagent was needed at all or whether, as implied above, it was possible to form an amide bond from direct reaction of a thioacid and amine.

Our first priority was to confirm the retention of stereochemical integrity at the *C*-terminal amino-acids during thioacid synthesis and the following peptide bond formation; for this, we looked to the literature for a simple, rapid method of identifying racemisation at this site.

3.2 ISOLEUCINE AS AN INDICATOR FOR *C*-TERMINAL EPIMERISATION

Epimerisation during peptide coupling is normally assessed by the preparation of model di- or tri-peptide sequences (e.g. H-Gly-L-Ser-L-Phe-NH_2, prepared using a solid phase Fmoc strategy), deprotection and separation of the epimerised product (H-Gly-D-Ser-L-Phe-NH_2) by HPLC, and comparison with a prepared standard.[22, 23] Alternatively, a sample of the final peptide can be hydrolysed and the constituent amino-acids reacted with a chiral derivitising agent, commonly Marfey's reagent.[24] Calculation of the amount of epimerised product can then be achieved by HPLC separation of the diastereomeric pairs.[25] ^{1}H, ^{13}C and ^{15}N NMR spectroscopy can also be used to calculate the level of epimerisation, either from identifying shifts caused by a mix of diastereomers or by association of the enantiomers with a chiral shift reagent.[26-28]

L-Isoleucine is one of 22 proteinogenic amino-acids found in eukaryotic and prokaryotic proteins,[29] but is unusual in that it possesses two stereocentres (Figure 26It can therefore exist as four distinct stereoisomers.[30]

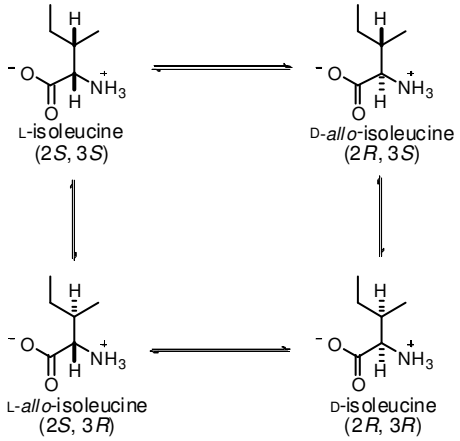


Figure 26: The 4 stereoisomers of isoleucine, showing the interconversion pathways for epimerisation at the α and β carbons

Epimerisation at the α-centre is relatively rapid due to resonance stabilisation and electron-withdrawing effects from the amino- and carboxy- group to give D-*allo*-isoleucine. In contrast, the β-centre is distanced from these effects and so is more difficult to epimerise to give the uncommon L-allo-isoleucine diastereomer.[30, 31] This lack of reactivity allows the β-configuration at the carbon to act as a marker for epimerisation at the α-carbon. Isoleucine as a marker for the study of racemisation during peptide synthesis has been known for a number of years,[23, 32, 33] but the final assessment of chiral purity is usually performed by hydrolysis followed by amino-acid analysis.[34]

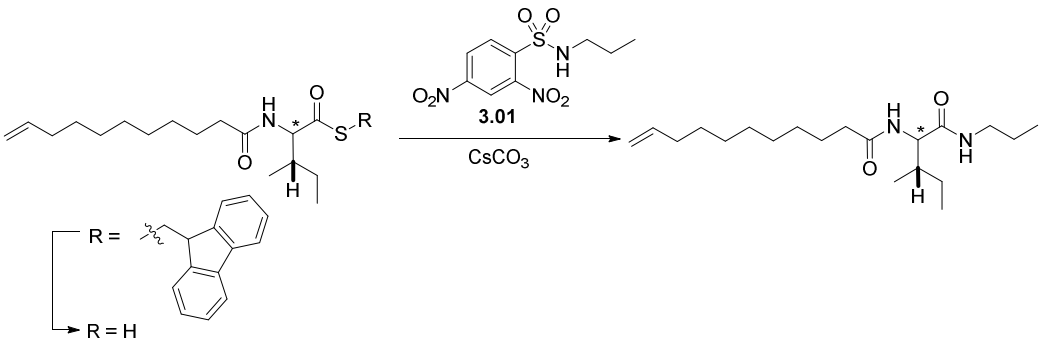
To our knowledge, only two references exist describing the differentiation of L-isoleucine and D-*allo*-isoleucine by ^{1}H NMR spectroscopy. In 1983, Khatskevich *et al.* reported that both the chemical shift and the coupling constants for the hydrogen at the α-carbon differed for *N*-benzoyl-L-isoleucine methyl ester and *N*-benzoyl-D-*allo*-isoleucine methyl ester ((2*S*, 3*S*) diastereomer, δ ppm 4.70, dd, $^{3}J_{CH\text{-}CH}$ 5.15 Hz, $^{3}J_{CH\text{-}NH}$ 8.62 Hz; ((2*R*, 3*S*) diastereomer, δ ppm 4.90, dd, $^{3}J_{CH\text{-}CH}$ 4.30 Hz, $^{3}J_{CH\text{-}NH}$ 9.0 Hz).[35] Similarly, in 2003 Biron and Kessler noted that, when hydrolysing N^{α}-methyl-N^{α}-*o*-NBS-L-isoleucine methyl ester, epimerisation at the α-stereocentre could be observed by the appearance of a new peak (corresponding to the D-*allo* diastereomer α-C<u>H</u>) downfield from the usual peak observed for the L- diastereomer.[36]

We hoped to exploit this distinction between the proton signals for L-isoleucine and its epimer, D-*allo*-isoleucine, to assess epimerisation of *C*-terminal during Crich's synthesis of thioacids, and their resulting amide-bond formation, compared to that occurring for the same peptide bond formed using the amino-acid and a coupling agent, PyBOP.[12] PyBOP (1H-benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate) has been shown to avoid epimerisation during thioester formation, particularly when combined with an excess of the thiol precursor and low reaction temperatures.[37]

3.3 INITIAL RESEARCH AIMS

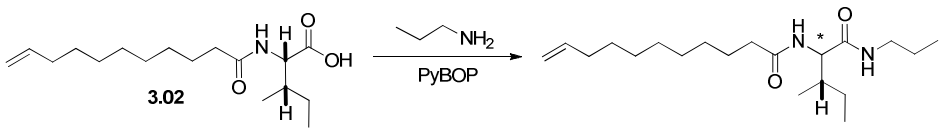
Undec-10-enoyl-L-isoleucine, **3.02** (Scheme 1) was chosen as the *C*-terminal amino-acid, and ^{n}propyl amine as the amide to give a very simple peptide with only one epimerisable stereocentre. We planned to synthesise this model peptide by two different routes:

1. Crich method of amide bond formation, *via* a thioacid



Scheme 10: The proposed route to the model peptide using Crich's method

2. A 'traditional' peptide coupling, according to Crich's procedure, but using a carboxylic acid rather than a thioacid

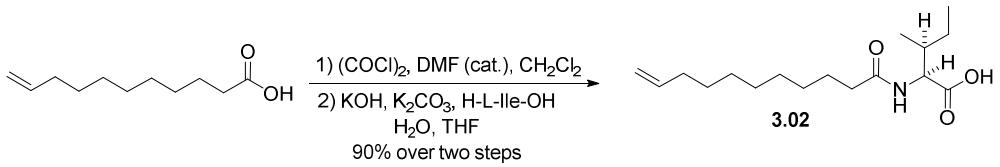


Scheme 11: The proposed route to the model peptide using a standard peptide coupling

Both reactions would be performed using undec-10-enoyl-L-isoleucine, **3.02**, and then repeated for comparison with a mix of undec-10-enoyl-L-isoleucine and undec-10-enoyl-D-*allo*-isoleucine, **3.03**. We hoped that the products would be sufficiently disparate by ^{1}H NMR spectroscopy to allow us to make an approximate measurement of the amount, if any, of epimerised product formed.

3.3.1 Synthesis and Epimerisation of Undec-10-enoyl-L-Isoleucine

Undec-10-enoyl-L-isoleucine was easily synthesised in two steps *via* the equivalent acid chloride (Scheme 12).



Scheme 12: The synthesis of undec-10-enoyl-L-isoleucine

In order to identify the appearance of the epimerised product by ^{1}H NMR , a sample of this was deliberately epimerised using acetic anhydride and aqueous sodium hydroxide, by the method of *du* Vigneaud and Meyer.[38] It seems likely that this promotes the formation of the 5-(4*H*)-oxazolone by forming the mixed anhydride, providing a good leaving group (Figure 27). An epimeric mixture of approximately 4:1 ratio of undec-10-enoyl-L-isoleucine and undec-10-enoyl-D-*allo*-isoleucine was obtained.

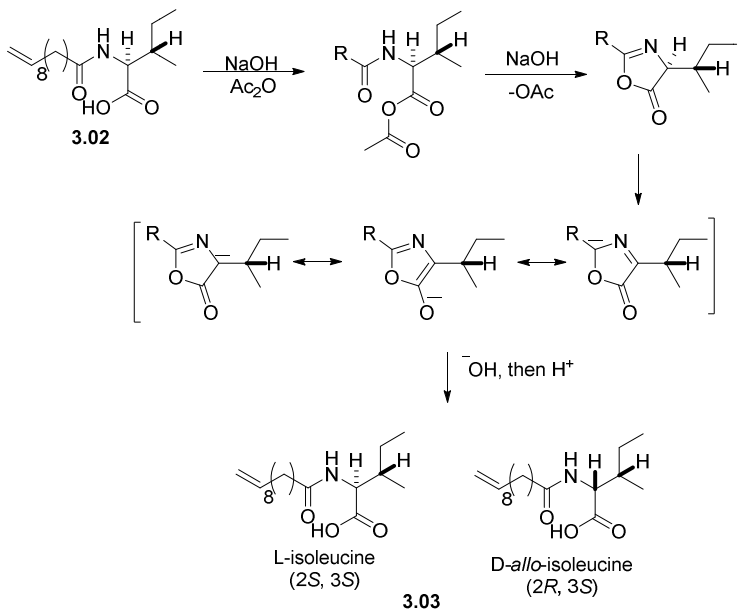


Figure 27: Epimerisation at α-CH of undec-10-enoyl-L-isoleucine by 5-(4*H*)-oxazolone formation

Pleasingly, comparison of the ^{1}H NMR spectra for the single diastereomer with that of the mix showed a noticeable difference in shift for the α-CH of L-isoleucine and D-*allo*-isoleucine (Figure 28).

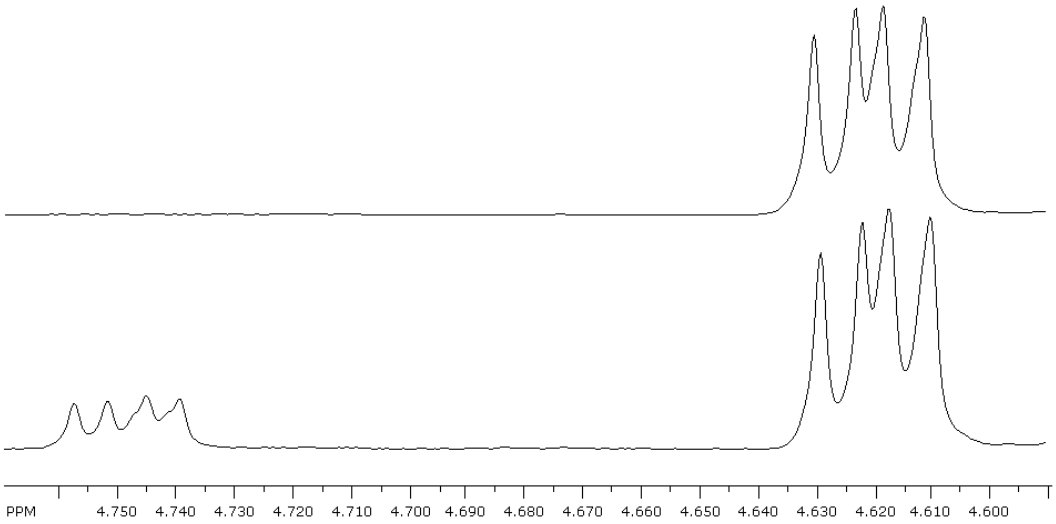
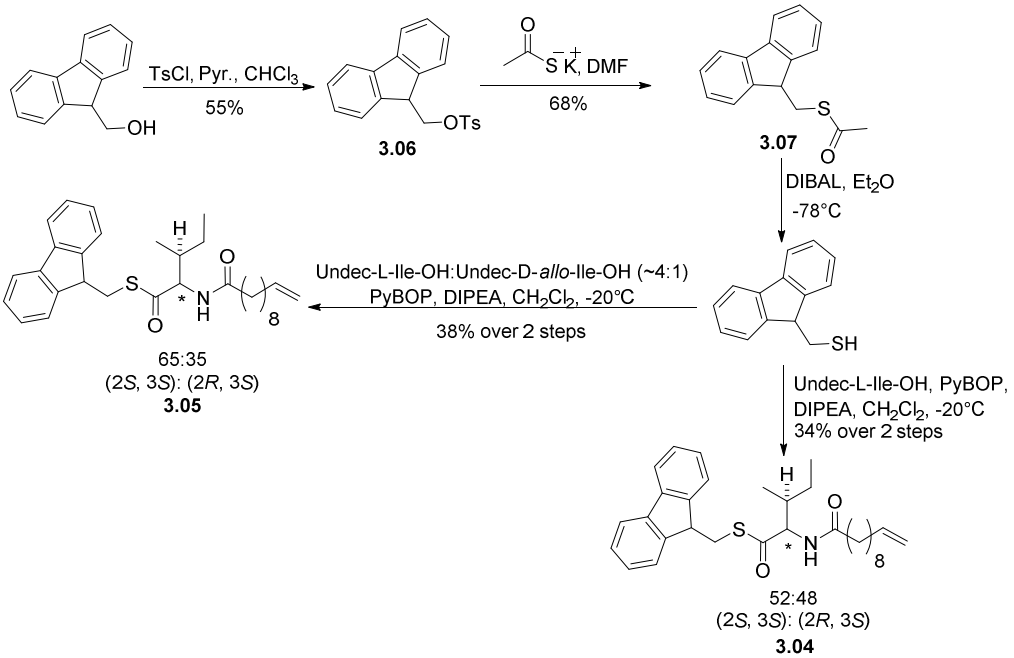


Figure 28: The dd caused by the α–CH of undec-10-enoyl-L-isoleucine, **3.02** (top spectrum) and the epimeric mixture **3.03**, bottom spectrum) of undec-10-enoyl-D-*allo*-isoleucine (around 4.74 ppm) and undec-10-enoyl-L-isoleucine (around 4.62 ppm)

3.3.2 CRICH AMIDE SYNTHESIS VIA THIOESTER

The fluorenylmethyl coupled thioester, **3.04**, was prepared according to Crich's method (Scheme 13).[5] The product from the coupling with the single diastereomer, undec-10-enoyl-L-isoleucine, **3.04**, showed that a significant proportion of the coupled thioester (39% of the crude product, 48% following purification by silica chromatography) was present as the D-*allo*-isoleucine diastereomer, shown by the presence of a new peak for the α-C<u>H</u> downfield from the L-diastereomer peak (Figure 29). This was confirmed by comparison with the coupled product formed using the 4:1 mix of L-isoleucine and D-*allo*-isoleucine diastereomers, **3.05**, (Scheme 13), which also showed a distinct peak for each diastereomer at the same chemical shifts (65% D-*allo* to 35% L-isoleucine for the crude product, 40% D-*allo*-isoleucine to 60% L-isoleucine following purification by silica chromatography).



Scheme 13: The synthetic route to the coupled fluorenyl-methylthioesters, from a single diastereomer and a mix of diastereomers of undec-10-enoyl-isoleucine

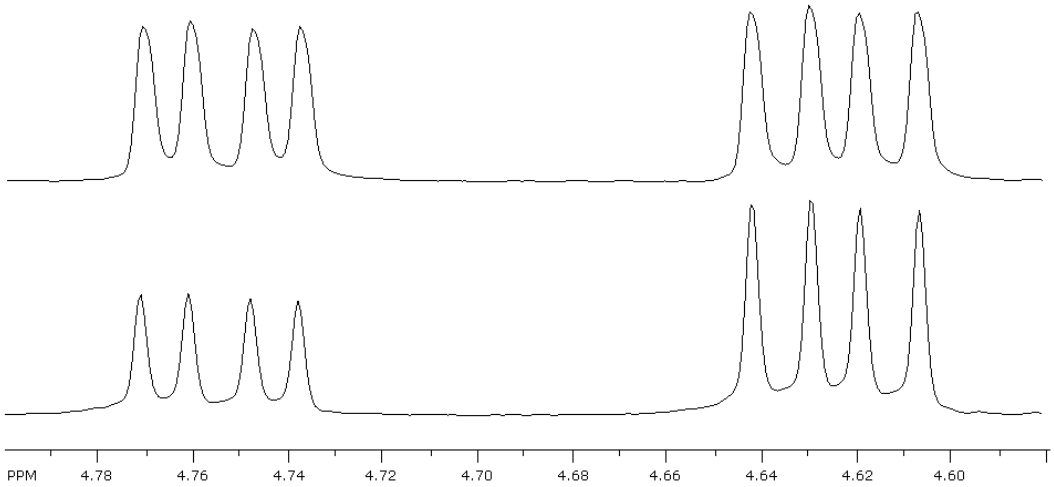
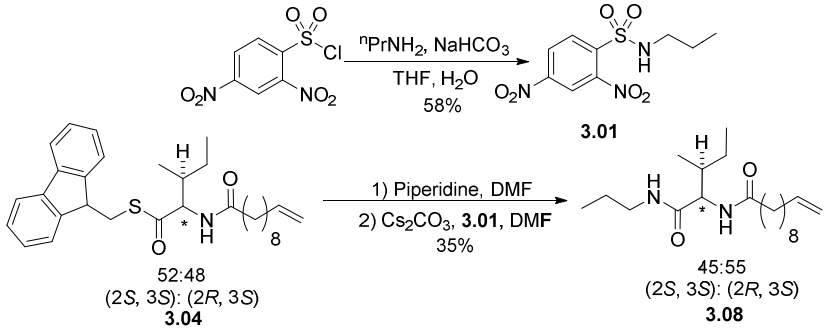


Figure 29: The dd peaks associated with the thioester α-C<u>H</u> signal, showing the signal from L-isoleucine at around 4.61 ppm and the signal from D-*allo*-isoleucine at around 4.74 ppm from the reaction with the single diastereomer, **3.04** (top spectrum), and the reaction with the mix of two diastereomers, **3.05** (bottom spectrum)

This was extremely concerning, as the literature for this method implied that extremely low levels of epimerisation were observed during the final peptide coupling, yet we were seeing near complete epimerisation of the *C*-terminal amino-acid even before release of the thioacid and amide bond formation. The thioester synthesis was repeated a further three times, but the levels of epimerisation observed were essentially the same.

The thioester **3.04** was then deprotected to give the thioacid and the model peptide **3.09** was synthesised using the sulfonamide of propylamine **3.01** (Scheme 14).

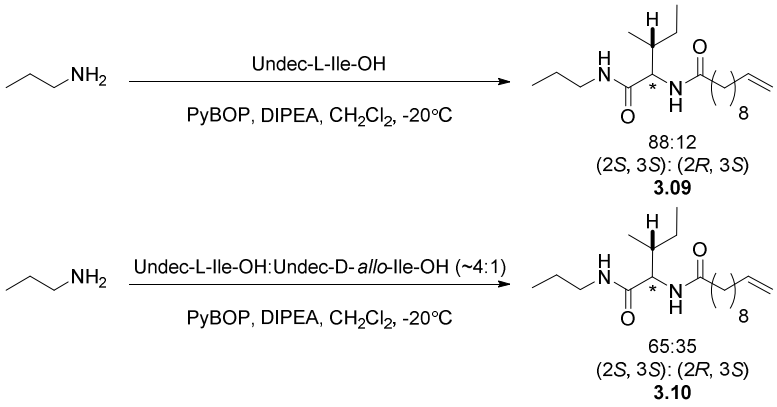


Scheme 14: The preparation of the model peptide **3.08** *via* the sulphonamide **3.01** and the thioester **3.04**

The characterisation data for this compound proved to be identical for that prepared by the standard PyBOP coupling (Scheme 15), but still showed ~1:1 mix of the L- and D-*allo*-isoleucine diastereomers.

3.3.3 PyBOP Amide Synthesis from Carboxylic Acid

In order to confirm that it was the formation of the thioester causing the epimerisation, we compared our results with the formation of the model peptides **3.09** and **3.10** using the peptide coupling agent PyBOP. As before, this was performed twice, once using a single diastereomer of undec-10-enoyl-L-isoleucine and once using a mix of undec-10-enoyl-L-isoleucine and undec-10-enoyl-D-*allo*-isoleucine (Scheme 15).

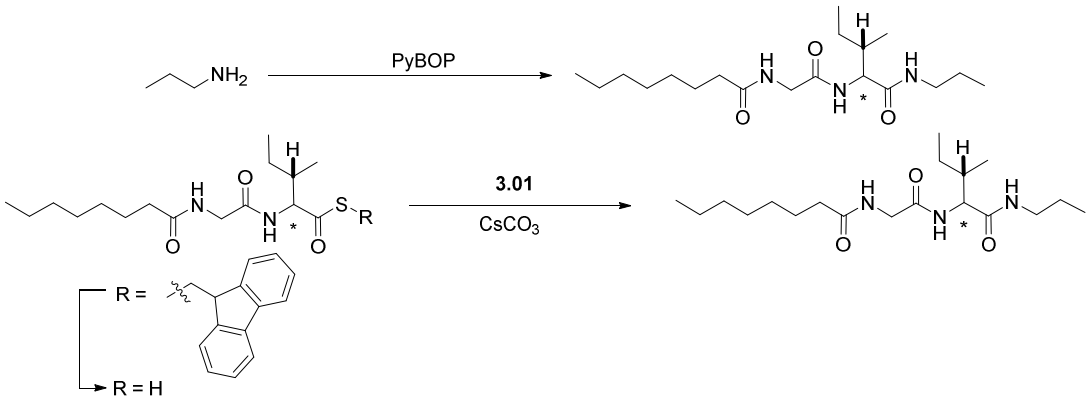


Scheme 15: Preparation of the model peptide, using the PyBOP strategy

Though significant epimerisation was observed during the coupling of the single diastereomer undecylenyl-L-isoleucine to the amine (14% D-*allo*-isoleucine observed in the crude reaction mixture, 12% following purification by silica chromatography for **3.10**), this was still much lower than the near complete epimerisation observed during the thioester formation.

3.4 PEPTIDE SYNTHESIS USING A MODEL DIPEPTIDE

This appeared to confirm that the first step of Crich's method, the formation of the thioester, was responsible for the extremely high levels of epimerisation observed in the model peptide. However, we were aware that *N*-terminal acylated amino-acids are more prone to racemisation than *N*-peptidyl amino-acids (e.g. dipeptides or longer), as the former would be expected to have a greater electron withdrawing effect on the *C*-terminal amide than the latter.[23, 39] We therefore investigated the levels of epimerisation on a more realistic peptide model, octanoyl-glycyl-L-isoleucine (Scheme 16).

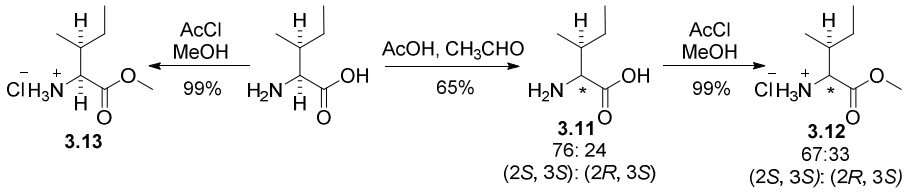


Scheme 16: The two strategies proposed for the preparation of the model dipeptide

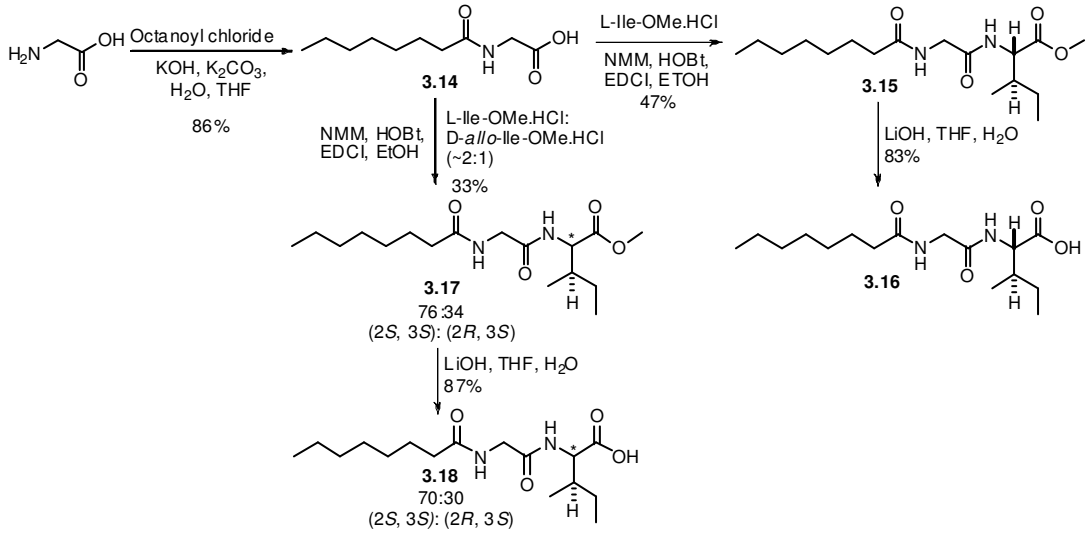
3.4.1 SYNTHESIS OF OCTANOYL-GLYCYL-ISOLEUCINE

In order to synthesise these compounds, we needed a method to produce a mixture of L-isoleucine and D-*allo*-isoleucine, without introducing an acyl group on the *N*-terminus. To do this, we followed the method of Yamada *et al*., which uses a substoichiometric quantity of an aldehyde in acetic acid to reversibly form an imine with the amino-acid nitrogen, followed by deprotonation at the α–centre by the acetate anion.[40] This provided a mixture of 24% D-*allo*-isoleucine and 76% L-isoleucine **3.11** which was converted to the methyl ester **3.12** (Scheme 17). These were then used for the synthesis of the model dipeptides **3.16** and **3.18** (Scheme 18). Again, we were able to discriminate between the CH peaks for octanoyl-glycyl-L-isoleucine and octanoyl-glycyl-D-*allo*-isoleucine by ^{1}H NMR spectroscopy.

Both the single diastereomer of octanoylglycyl-L-isoleucine, **3.16**, and the mix of diastereomers, **3.18**, were used for the synthesis of the fluorenylmethyl thioesters **3.19** and **3.20** respectively (Scheme 19) and, for comparison, the propylamides (**3.22** and **3.23** respectively (Scheme 20).



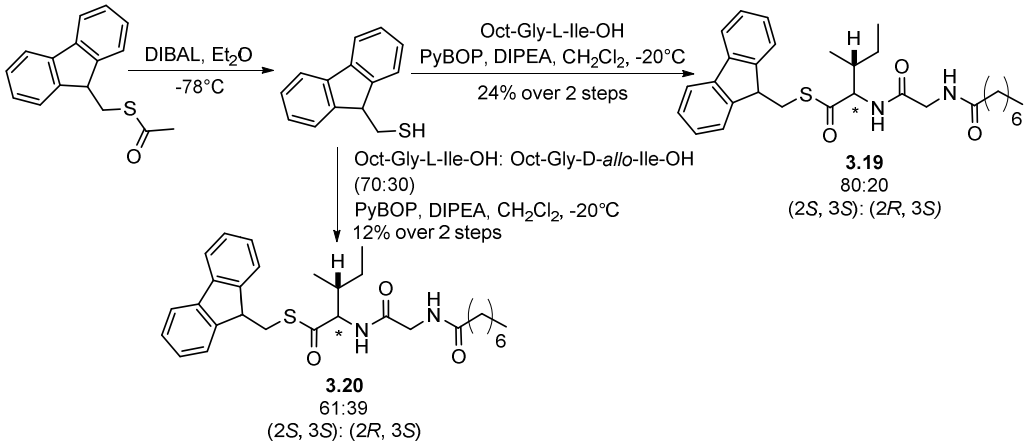
Scheme 17: The synthesis of isoleucine methyl ester, as both the single diastereomer and a mix of L-isoleucine and D-*allo*-isoleucine diastereomers



Scheme 18: The synthesis of octanoyl-glycyl-isoleucine from the single diastereomer of isoleucine methyl ester and from a mix of L- and D-*allo*-isoleucine diastereomers

3.4.2 MODEL DIPEPTIDE SYNTHESIS USING THIOESTERS

The synthesis of the two thioesters was performed as before and again, significant epimerisation of the *C*-terminal-L-isoleucine residue (12 % D-*allo* to 88 % L-isoleucine for the crude product, 20 % D-*allo*-isoleucine to 80 % L-isoleucine following purification by silica chromatography) was still observed for the thioester formation (Scheme 19).



Scheme 19: The synthesis of the dipeptide thioesters from the single diastereomer of octanoylglycyl isoleucine and the mix of L- and D-*allo*- diastereomers

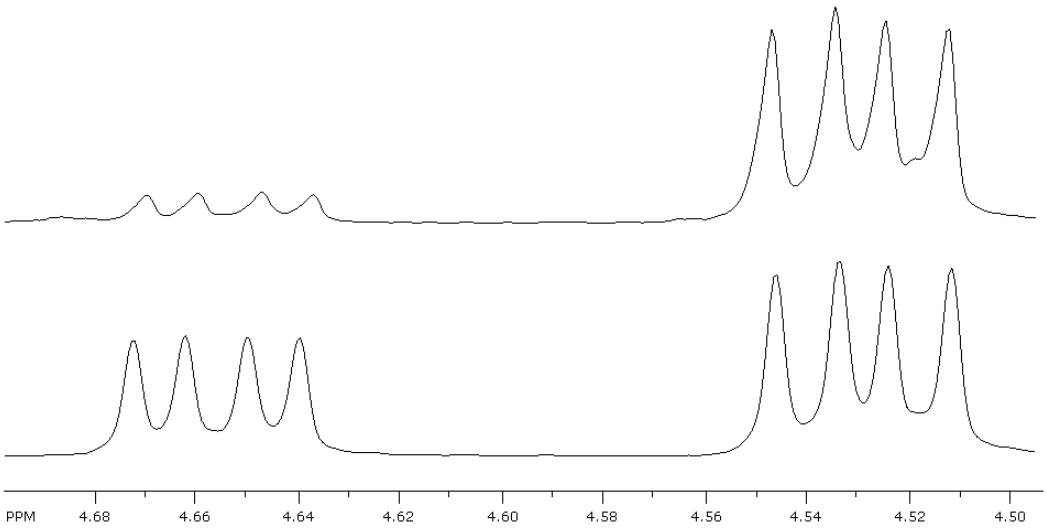
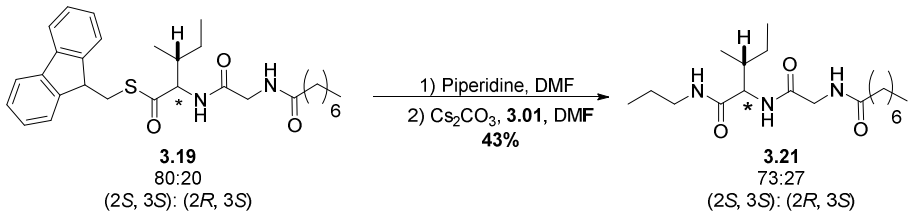


Figure 30: The dd peaks associated with the thioester α-CH signal, showing the signal from L-isoleucine derivative at around 4.54 ppm and the signal from D-*allo*-isoleucine derivative at around 4.67 ppm from the reaction with the single diastereomer **3.19** (top spectrum) and the reaction with the mix of two diastereomers **3.20** (bottom spectrum)

As expected, the degree of epimerisation was lower than observed for the undecylenylisoleucine products, as the glycine amide carbonyl of the dipeptide is less nucleophilic than the equivalent acyl amide carbonyl, due to the presence of an electron withdrawing CONH at the α–carbon, rather than the relatively electron-donating simple alkyl chain of undecylenylisoleucine .

Again, the thioester was deprotected and coupled with sulphonamide **3.01** to give the model dipeptide **3.21** (Scheme 20). This showed comparable levels of epimerisation

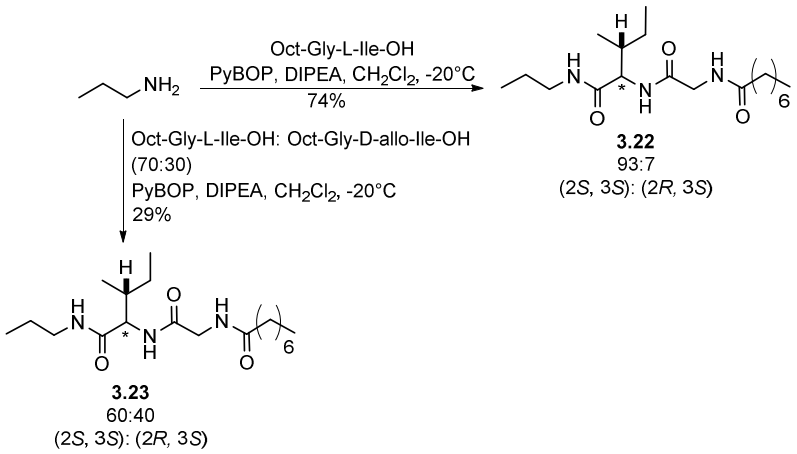
at the isoleucine α-CH, with around 27 % epimer present in the purified product (27 % D-*allo*-isoleucine derivative present before purification, judged by ^{1}H NMR). This appears to confirm that the epimerisation occurs during the initial thioester formation, rather than during the peptide coupling step.



Scheme 20: The formation of the model dipeptide from thioester **3.19**

3.4.3 Model Dipeptide Synthesis using PyBOP

As before, the equivalent peptide coupling reaction, performed using the single diastereomer of carboxylic acid to give the propyl amide **3.22** (Scheme 21), showed significantly lower levels of epimerised product (~7% D-*allo*-isoleucine by ^{1}H NMR spectroscopy, Figure 31).



Scheme 21: The synthesis of the dipeptide propylamines **3.22** and **3.23**

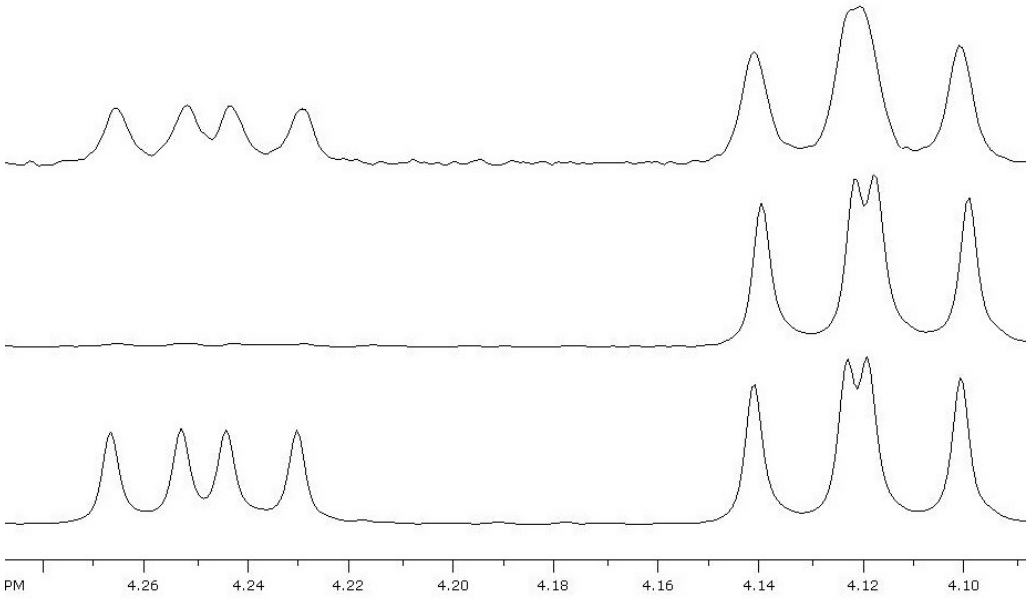


Figure 31: The dd peaks associated with the amide α-CH signal, showing the signal from L-isoleucine derivative at around 4.12 ppm and the signal from D-*allo*-isoleucine derivative at around 4.25 ppm. The top spectrum shows the dipeptide **3.21** from the thioester **3.19**, the middle spectrum **3.22** from the PyBOP coupling of reaction with the single diastereomer and the bottom spectrum shows **3.23** from the reaction with the mix of two diastereomers

3.5 CONCLUSIONS

From these reactions, we concluded that, at least in terms of epimerisation, the Crich method of thioester synthesis, followed by thioacid formation and amide bond synthesis, was significantly worse than the equivalent peptide coupling reaction using just the phosphonium salt. It appears that the initial thioester formation was much slower than the equivalent peptide coupling, leading to a much greater risk of epimerisation by 5-(4*H*)-oxazolone formation. We therefore abandoned this thioester precursor as a new method for the formation of CTLPs or other peptides.

In constrast, we were extremely interested by the reliability and predictability by which D-*allo*-isoleucine and L-isoleucine could be distinguished by ^{1}H NMR. We noted that there was a consistent trend in the relative shifts of the α-CH, as well as the coupling constants for these diastereomers, with the D-*allo*-diastereomer appearing downfield to the L-diastereomer. For our purposes, this had served as an

extremely useful method of assessing levels of epimerisation. It should be stated however, that the sensitivity of this method has not been fully explored, though we expect it to be accurate to <5%. As such, it proved to be a valuable tool to rapidly estimate the chiral integrity of a *C*-terminal isoleucine and, while it may lack the sensitivity of HPLC methods, it has the potential to be an extremely useful tool for the comparison for a number of peptide coupling methods.

A further, potentially more valuable, function of this spectroscopic occurrence is to allow the differentiation of isoleucine diastereomers in natural products.

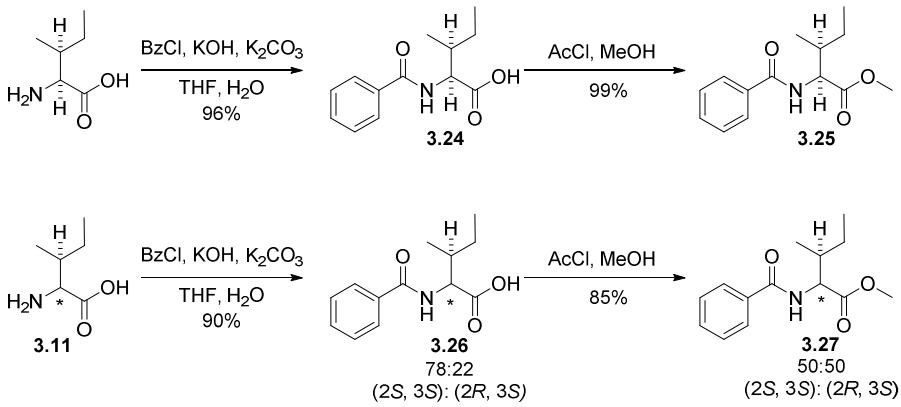
3.6 Trends in the ^{1}H NMR Spectra of Two Diastereomers of Isoleucine

While L-isoleucine is the most common diastereomer in natural products, D-*allo*-isoleucine is also fairly common in microbial peptides, including the sporidemolides, stendomycin, angolide, actinomycin C and peptidolipin N.A.[41] In addition, D-*allo*-isoleucine has been identified in peptides isolated from the skin of the yellow bellied toad, *Bombina variegate*.[42] Unsurprisingly, considering the comparative stability of the β-C<u>H</u> of isoleucine (*vide supra*), peptides containing L-*allo*-isoleucine and D-isoleucine are much more uncommon, though a few examples do exist. Among these are the bacterial peptide, cypemycin, which contains two L-*allo*-isoleucine residues that occur during post-translational modification.[43] D-Isoleucine is found in the bacterial peptide monocycin,[44] while some members of the actinomycin family of *Streptomyces* derived peptides are known to contain both D-isoleucine and *N*-methyl-L-isoleucine.[45, 46] It is also to be noted that, in the case of the metabolic disorder, maple syrup urine disease, L-*allo*-isoleucine can be produced by the body, *via* keto-formation at the α-centre of L-isoleucine.[47]

The ability to distinguish between the isoleucine diastereoisomers without the hydrolysis of the parent protein would be valuable, particularly for complex natural proteins, which are often only obtained in minute quantities. A method to distinguish between diastereomers by ^{1}H NMR spectroscopy would therefore be of considerable use as it is non-destructive. While we cannot distinguish between L-*allo*- and D-*allo*-, and between the L- and D- isoleucine diastereomers, the comparative rarity of the D- and L-*allo* forms mean that this method could still have utility.

We investigated whether the trend noted during the synthesis of the isoleucyl thioesters would prove a reliable method for differentiating L-isoleucine from D-*allo*-isoleucine by ^{1}H NMR within the native peptide. In addition to the compounds already synthesized (Figure 32), we included two more compounds, benzoyl-

isoleucine and benzoyl-isoleucine methyl ester (**3.24 - 3.27**, Scheme 22), as both the single and mix of diastereomers.



Scheme 22: The synthesis of benzoyl-isoleucine and benzoyl-isoleucine methyl ester as both the single and the mix of two diastereomers, for analysis by ^{1}H NMR spectroscopy

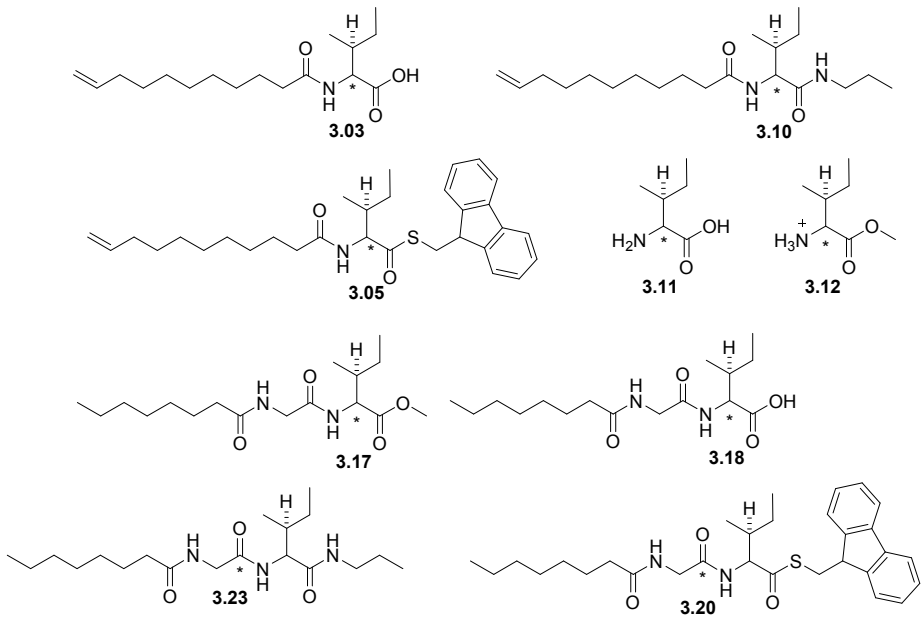


Figure 32: The previously synthesised structures used for analysis by ^{1}H NMR spectroscopy

We then obtained the ^{1}H NMR spectroscopic data for these 11 compounds, both as a mix of diastereomers and, where possible, as the single diastereomer. The data was obtained in three deuterated solvents ($CDCl_3$, DMSO-d_6, and CD_3OD). The spectra were obtained on 700 MHz NMR machines, and, after calibration against the internal solvent peak, the chemical shifts and coupling constants for the α–C<u>H</u> were

measured. Where spectra for the both the single diastereomer and the mix were obtained, the values for the L-diastereomer were averaged from the two.

^{13}C NMR data was also obtained for most of these compounds on the 400 MHz NMR machine, though in $CDCl_3$ only. The chemical shifts corresponding to the α–*C*H of were measured.

The results are shown below.

Compound	L-isoleucine diastereomer				D-*allo*-isoleucine diastereomer			
	α-C*H* (ppm)	$^3J_{CH\text{-}CH}$ (Hz)	$^3J_{CH\text{-}NH}$ (Hz)	α-*C*H (ppm)	α-C*H* (ppm)	$^3J_{CH\text{-}CH}$ (Hz)	$^3J_{CH\text{-}NH}$ (Hz)	α-*C*H (ppm)
3.03	4.62	5.1	8.0	56.5	4.75	4.0	8.6	55.2
3.05	4.63	5.1	8.2	63.2	4.76	4.0	9.1	61.8
3.10	4.21	7.9	7.9	57.5	4.32	6.6	8.4	57.0
3.11	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
3.12	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
3.17	4.54	5.3	8.4	56.7	4.65	4.1	8.8	55.6
3.18	4.53	5.3	7.5	56.9	4.69	4.5	8.5	n/a
3.20	4.53	4.8	8.8	63.8	4.66	4.0	8.8	62.3
3.23	4.34	7.5	7.5	56.7	4.45	5.7	8.8	55.6
3.26	4.86	4.9	8.1	57.0	4.98	3.9	8.5	55.7
3.27	4.84	5.2	8.4	56.8	4.95	4.0	8.8	55.8

Table 2: Chemical shifts and coupling constants in $CDCl_3$.

Compound	L-isoleucine diastereomer		D-*allo*-isoleucine diastereomer	
	α-C<u>H</u> shift (ppm)	$^3J_{CH\text{-}CH}$ (Hz)	α-C<u>H</u> shift (ppm)	$^3J_{CH\text{-}CH}$ (Hz)
3.03	4.37	5.8	4.54	4.7
3.05	4.31	6.1	4.48	5.1
3.10	4.15	8.3	4.31	6.7
3.11	3.47	3.9	3.53	3.6
3.12	4.00	4.0	4.03	3.9
3.17	4.42	6.0	4.56	4.4
3.18	4.40	5.3	4.55	4.0
3.20	4.33	5.7	4.48	4.8
3.23	4.20	7.5	4.35	5.7
3.26	4.57	6.4	4.74	5.0
3.27	4.56	6.9	4.74	5.6

Table 3: Chemical shifts and coupling constants in CD_3OD

Compound	L-isoleucine diastereomer			D-*allo*-isoleucine diastereomer		
	α-C<u>H</u> shift (ppm)	$^3J_{CH\text{-}CH}$ (Hz)	$^3J_{CH\text{-}NH}$ (Hz)	α-C<u>H</u> shift (ppm)	$^3J_{CH\text{-}CH}$ (Hz)	$^3J_{CH\text{-}NH}$ (Hz)
3.03	4.17	6.5	8.1	4.35	5.0	8.5
3.05	4.15	7.4	7.4	4.33	5.1	8.7
3.10	4.10	8.5	8.5	4.25	6.3	9.0
3.11	3.05	3.2	n/a	3.08	2.9	n/a
3.12	3.91	4.2	n/a	3.92	3.8	n/a
3.17	4.23	6.6	8.4	4.39	5.1	8.6
3.18	4.19	5.9	8.6	4.36	4.4	8.8
3.20	4.15	6.2	8.4	4.33	5.1	8.6
3.23	4.12	7.5	8.8	4.25	5.7	9.2
3.26	4.33	7.6	7.6	4.53	5.8	8.5
3.27	4.36	7.7	7.7	4.55	6.4	8.0

Table 4: Chemical shifts and coupling constants in DMSO-d_6

The general trends noted earlier were further supported by these results. In summary we found that, in the three solvents tested:

- The chemical shift for the D-*allo*-isoleucine diastereomer always occurred at a higher ppm than that for the L-isoleucine diastereomer
- The $^{3}J_{CH\text{-}CH}$ coupling constants for the L-isoleucine diastereomer α-C<u>H</u> were always larger than those for the D-*allo*-isoleucine diastereomer
- The $^{3}J_{CH\text{-}NH}$ coupling constants for the L-isoleucine diastereomer α-C<u>H</u> were always smaller than those for the D-*allo*-isoleucine diastereomer

Generally, the difference in chemical shift was greatest in DMSO-d_6, then CD_3OD and smallest in $CDCl_3$, but the two peaks observed were always sufficiently distinct not to overlap. This suggests they would be suitable for the approximate assignment of relative stereochemistry of isoleucine residues in novel peptides.

For some compounds, namely the two thioesters, **3.05** and **3.27,** the diastereomers were sufficiently different in both DMSO-d_6 and CD_3OD for other peaks to be well separated and identified by ^{1}H NMR spectroscopy. The doublet and triplet shifts corresponding to the terminal methyl groups of the isoleucine sidechain were particularly obvious.

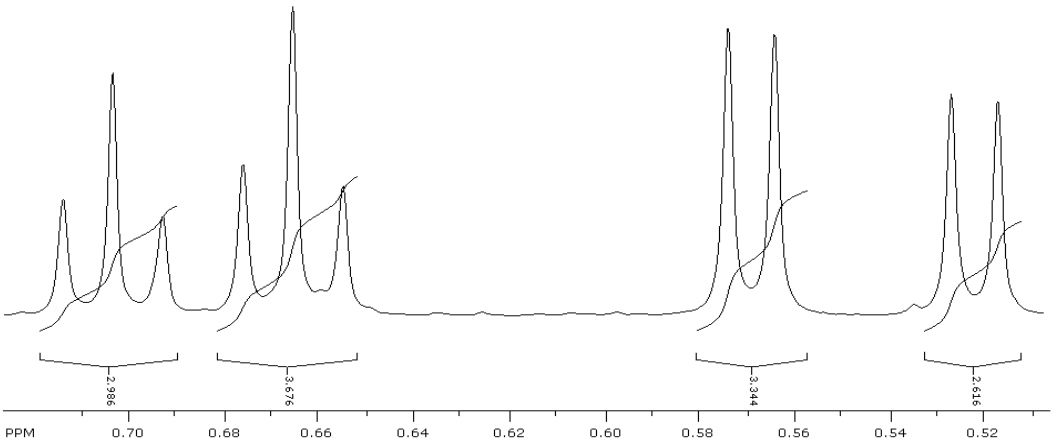


Figure 33: From **3.20**, chemical shifts for CH_2CH_3 for the D-*allo*-isoleucine derivative (~0.70 ppm) and the L-isoleucine derivative (~0.66 ppm) and $CHCH_3$ for the L-isoleucine derivative (~0.57 ppm) and the D-*allo*-isoleucine derivative (~0.52 ppm).

However, this was not common to all the compounds tested, so would be unsuitable for the general identification of the diastereomers.

Though the data obtained from the ^{13}C NMR spectra was less complete than for the ^{1}H NMR specta, the measurements obtained also suggest a useful trend. The chemical shift corresponding to the α-CH of the D-*allo*-isoleucine derivative always occurred at a lower ppm than that for the L-isoleucine derivative, in most cases by more than 1 ppm. Taken in conjunction with the ^{1}H NMR data, the ^{13}C NMR data is also likely to prove extremely useful for the approximate assignment of relative stereochemistry of isoleucine residues in novel peptides.

3.7 REFERENCES

1. T. Rosen, I. M. Lico and D. T. W. Chu, *J. Org. Chem.*, 1988, **53**, 1580-1582.
2. N. Shangguan, S. Katukojvala, R. Greenberg and L. J. Williams, *J. Am. Chem. Soc.*, 2003, **125**, 7754-7755.
3. Y. Yuan, J. Zhu, X. Li, X. Wu and S. J. Danishefsky, *Tetrahedron Lett.*, 2009, **50**, 2329-2333.
4. D. Crich and K. Sasaki, *Org. Lett.*, 2009, **11**, 3514-3517.
5. D. Crich, K. Sana and S. Guo, *Org. Lett.*, 2007, **9**, 4423-4426.
6. D. Crich and I. Sharma, *Angew. Chem., Int. Ed.*, 2009, **48**, 7591-7594.
7. T. Messeri, D. D. Sternbach and N. C. O. Tomkinson, *Tetrahedron Lett.*, 1998, **39**, 1669-1672.
8. T. Messeri, D. D. Sternbach and N. C. O. Tomkinson, *Tetrahedron Lett.*, 1998, **39**, 1673-1676.
9. V. R. Pattabiraman and J. W. Bode, *Nature*, 2011, **480**, 471-479.
10. R. P. Smith and R. E. Gosselin, *J. Occup. Med.*, 1979, **21**, 93-97.
11. P. Loeliger and E. Flückiger, *Org. Synth.*, 1976, 127-127.
12. D. Crich and I. Sharma, *Angew. Chem., Int. Ed.*, 2009, **48**, 2355-2358.
13. D. Crich and K. Sana, *J. Org. Chem.*, 2009, **74**, 7383-7388.
14. K. Sasaki and D. Crich, *Org. Lett.*, 2010, **12**, 3254-3257.

15. P. Wang and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2010, **132**, 17045-17051.
16. C.-F. Liu, C. Rao and J. P. Tam, *Tetrahedron Lett.*, 1996, **37**, 933-936.
17. J. C. Sheehan and D. A. Johnson, *J. Am. Chem. Soc.*, 1952, **74**, 4726-4727.
18. D. Yamashiro and J. Blake, *Int. J. Pept. Protein Res.*, 1981, **18**, 383-392.
19. R. Liu and L. E. Orgel, *Nature*, 1997, **389**, 52-54.
20. P. Botti, M. Villain, S. Manganiello and H. Gaertner, *Org. Lett.*, 2004, **6**, 4861-4864.
21. P. Agrigento, F. Albericio, S. Chamoin, I. Dacquignies, H. Koc and M. Eberle, *Org. Lett.*, 2014.
22. A. Di Fenza, M. Tancredi, C. Galoppini and P. Rovero, *Tetrahedron Lett.*, 1998, **39**, 8529-8532.
23. E. Gross and J. Meienhofer, *The Peptides: Major Methods of Peptide Bond Formation*, Academic Press, 1979.
24. P. Marfey, *Carlsberg Res. Commun.*, 1984, **49**, 591-596.
25. R. Bhushan and H. Brückner, *Amino Acids*, 2004, **27**, 231-247.
26. E. D. Gómez and H. Duddeck, *Magn. Reson. Chem.*, 2009, **47**, 222-227.
27. H. R. Kricheldorf and W. E. Hull, *Org. Magn. Reson.*, 1979, **12**, 607-611.
28. B. Weinstein and A. E. Pritchard, *J. Chem. Soc., Perkin Trans. 1*, 1972, 1015-1020.
29. B. Hao, W. Gong, T. K. Ferguson, C. M. James, J. A. Krzycki and M. K. Chan, *Science*, 2002, **296**, 1462-1466.
30. J. L. Bada, M. Zhao, S. Steinberg and E. Ruth, *Nature*, 1986, **319**, 314-316.
31. M. Bodanszky and D. Perlman, *Nature*, 1968, 291-292.
32. M. Bodanszky and L. E. Conklin, *Chem. Commun. (London)*, 1967, 773-774.
33. M. Itoh, *Bull. Chem. Soc. Jpn.*, 1973, **46**, 2219-2221.
34. D. H. Spackman, W. H. Stein and S. Moore, *Anal. Chem.*, 1958, **30**, 1190-1206.
35. I. É. Khatskevich, I. K. Kalnin, E. I. Karpeiskaya and E. I. Klabunovskii, *Bull. Acad. Sci. USSR, Div. Chem. Sci. (Engl. Transl.)*, 1983, **32**, 323-329.

36. E. Biron and H. Kessler, *J. Org. Chem.*, 2005, **70**, 5183-5189.

37. Y. Kajihara, A. Yoshihara, K. Hirano and N. Yamamoto, *Carbohydr. Res.*, 2006, **341**, 1333-1340.

38. V. du Vigneaud and C. E. Meyer, *J. Biol. Chem.*, 1932, **98**, 295-308.

39. B. Halpern, L. F. Chew and B. Weinstein, *J. Am. Chem. Soc.*, 1967, **89**, 5051-5052.

40. S. Yamada, C. Hongo, R. Yoshioka and I. Chibata, *J. Org. Chem.*, 1983, **48**, 843-846.

41. M. Bodanszky and D. Periman, *Science*, 1969, **163**, 352-358.

42. G. Mignogna, M. Simmaco, G. Kreil and D. Barra, *EMBO J.*, 1993, **12**, 4829-4832.

43. J. Claesen and M. Bibb, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 16297-16302.

44. K. Bevan, J. S. Davies, C. H. Hassall and D. A. S. Phillips, *J. Chem. Soc. D*, 1969, 1246a-1246a.

45. T. Yajima, K. Mason and E. Katz, *Antimicrob. Agents Chemother.*, 1976, **9**, 224-232.

46. T. Yajima, K. T. Mason and E. Kaltz, *Antimicrob. Agents Chemother.*, 1975, **7**, 773-780.

47. S. E. Snyderman, P. M. Norton, E. Roitman and L. E. Holt, *Pediatrics*, 1964, **34**, 454-472.

3.8 EXPERIMENTAL

All the reagents and solvents used were purchased from the Sigma-Aldrich, Alfa-Aesar, TCI, Bachem or Fluorochem Chemical Company and were used as received unless stated otherwise. pH 2 buffer was made as a solution of 0.75 M of Na_2SO_4 and 0.25 M H_2SO_4 in H_2O.

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AVII-700 MHz, AVIII-600 MHz, DRX-500 MHz or DPX-400 MHz Fourier transform spectrometer at room temperature unless stated otherwise. Chemical shifts are quoted in parts per million (ppm) downfield from tetramethylsilane. Solvents were used as an internal standard when assigning NMR spectra (δ_H: $CDCl_3$ 7.26 ppm, CD_3OD 3.31 ppm, d_6 -DMSO 2.50 ppm, D_2O 4.79 ppm; δ_C: $CDCl_3$ 77.1 ppm, CD_3OD 49.0 ppm, d_6 -DMSO 39.5 ppm). Coupling constants (*J*) are quoted in Hertz (Hz). Coupling constants are rounded to the nearest 0.5 Hz for spectra recorded on the AVIII-600 MHz, DRX-500 MHz or DPX-400 MHz machines. Coupling constants recorded on the AVII-700 MHz machine are rounded to the nearest 0.1 Hz. Abbreviations used in the descriptions of spectra are as follows; s = singlet, d = doublet, t = triplet, q = quartet, quin. = quintet, sept. = septet, oct. = octet, m = multiplet, br = broad, i = ipso, o = ortho, m = meta, p = para, ax. = axial and eq. = equatorial. ^{13}C-NMR spectra were recorded with broadband proton decoupling and spectra were assigned on the basis of COSY, PENDANT, HMQC and HMBC spectra. In aromatic characterisations, the ipso carbon is taken to be the carbon bonded to the group with the highest molecular weight

Infrared spectra were recorded on a Nicolet Avatar 320 FT-IR spectrophotometer using EZ OMNIC software package 1, Bruker ALPHA Platinum ATR

spectrophotometer or Perkin ELMER Spectrum 100 FT-IR spectrophotometer using OPUS software and are quoted in wavenumber (cm^{-1}).

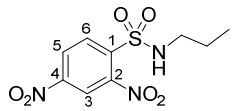
Optical rotations were recorded on an Optical Activity Ltd. AA-1000 millidegree auto-ranging polarimeter (using the sodium D line; 589 nm) and $[\alpha]_D$s are given in units of 10^{-1}deg cm^2 g^{-1}. The samples were made using spectroscopic grade MeOH, $CHCl_3$ or H_2O.

ESI mass spectra were obtained on a Bruker Esquire 2000 mass spectrometer or an Agilent 6130B single Quad (ESI)**.** HRMS ESI spectra were obtained by Dr Lijiang Song, Mr Philip Aston or Dr Rebecca Wills using a Bruker micro-TOF ESI attached to a time of flight (TOF) analyser.

Melting points for solid crystalline products were determined on a Stuart Scientific SMP10 Digital Melting Point Apparatus, with three runs of each compound, and a range given in ˚C rounded to the nearest degree. They are uncorrected. CHN elemental analyses were carried out by Warwick Analytical Services.

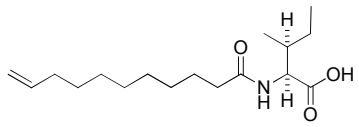
Thin Layer Chromatography (TLC) was performed using silica (0.25 mm) coated aluminium plates.

2, 4-Dinitro-*N*-propylbenzenesulfoniamide 3.01



Method modified from literature procedure by Crich *et al.*[1] A solution of 2,4-dinitrophenyl sulfonyl chloride (3.0 g, 11.3 mmol) in THF (30 mL) was added to an ice-cold solution of $NaHCO_3$ (2.84 g, 33.8 mmol) and *n*-propylamine (0.93 mL, 11.3 mmol) in THF: water (1:1, 72 mL). The reaction mixture was stirred for 3 hours at room temperature before acidification with pH 2 buffer. The reaction mixture was poured into EtOAc (200 mL) and the phases were separated. The organic phase was washed with pH 2 buffer (100 mL), water (100 mL) and saturated aqueous NaCl solution (100 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by silica chromatography ($CHCl_3$) gave the sulfonamide as a bright yellow oil that crystallised on standing to give **3.01** as a yellow crystalline solid (1.9 g, 6.6 mmol, 58 %); m.p. 78 - 80 °C; υ_{max} /cm^{-1} (neat) 3330 (N-H), 1550, 1534 (S=O), 1347, 1337(N=O); δ_H (400 MHz, $CDCl_3$) 8.68 (1H, d, *J* 2.5 Hz, C3-*H*), 8.57 (1H, dd, *J* 8.5, 2.5 Hz, C5-*H*), 8.37 (1H, d, *J* 8.5 Hz, C6-*H*), 5.35 (1H, t, *J* 6.0 Hz, N*H*CH_2), 3.14 (2H, td, *J* 7.0, 6.0 Hz, NHC*H*$_2$), 1.58 (2H, sxt, *J* 7.5 Hz, $NHCH_2CH_2$), 0.93 (3H, t, *J* 7.5 Hz, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 149.8, 139.5 (*C-1*, *C-2*, *C-4*), 132.6 (*C6*), 127.1 (*C5*), 120.2 (*C3*), 45.8 (NH*C*H_2), 23.1 ($NHCH_2CH_2$), 11.0 (*C*H_3); *m/z* (ESI+) 311.9 ([M+Na], 100%); HR-ESIMS: calculated for $C_9H_{11}N_3O_6SNa$: 312.0261, found 312.0264 $[M+Na]^+$.

(3*S*, 2*S*)-2-(Undec-10'-enoylamino)-3-methyl-pentanoic acid 3.02

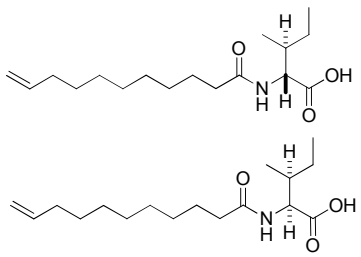


10-Undecenoic acid (3.00 g, 16.3 mmol) and DMF (3 drops) were stirred on ice in CH_2Cl_2 (50 mL) to give a yellow solution. Oxalyl chloride (4.13 g, 32.6 mmol) was added dropwise with exotherm and the reaction was allowed to reach room temperature over 1 hour. The reaction mixture was concentrated *in vacuo* to give the crude acid chloride as a brown oil, which was used immediately without further purification (3.20 g, 15.7 mmol, 97%); υ_{max}/cm^{-1} 2926 (CH), 2856 (CH), 1798 (C=O), 1463 (C=C); δ_H (400 MHz, $CDCl_3$) 5.83 (1H, ddt, *J* 17.0, 10.0, 6.5 Hz, CH_2=C*H*), 5.05 - 4.93 (2H, m, CH_2=CH), 2.91 (2H, t, *J* 7.5 Hz, CH_2COCl), 2.10 - 2.01 (2H, m, CH_2=CHCH_2), 1.73 (2H, quin, *J* 7.5 Hz, C$H_2$$CH_2$COCl), 1.49 - 1.23 (12H, m, 6 x CH_2); δ_C (100 MHz, $CDCl_3$) 173.8 (*C*OCl), 139.1 (CH_2=*C*H), 114.2 (*C*H_2=CH), 47.1 (*C*H_2COCl), 33.8 (*C*$H_2$$CH_2$COCl), 29.2, 29.0, 28.9, 28.5, 28.4, 25.1 (6 x CH_2); *m/z* (ESI+) 167.2 ([M-Cl]+, 80.6%); HR-ESIMS: calculated for $C_{11}H_{19}O$: 167.1430, found 167.1430 $[M-Cl]^+$. Data are consistent with that previously reported.[2]

A solution of the 10-undecenoyl chloride (5.10 g, 25 mmol) in THF (25 mL) was added over 5 minutes to a stirred solution of L-isoleucine (3.94 g, 30 mmol), potassium carbonate (1.68 g, 30 mmol) and potassium hydroxide (4.15 g, 30 mmol) in water (60 mL) at room temperature. The reaction mixture was stirred at room temperature for 18 hours. The mixture was acidified with pH 2 buffer and reduced *in vacuo* to give a white precipitate. The precipitate was isolated by vacuum filtration and the filtercake was washed successively with water (2 x 50 mL) and petroleum ether (3 x 50 mL) before drying by toluene azeotrope to afford the product as a bright white crystalline solid (6.71 g, 22.6 mmol, 90 %); m.p. 99 - 100 °C (lit.[3] 100 - 101 °C); $[\alpha]_D^{27}$ +20.6 (c = 1.0, $CHCl_3$), $[\alpha]_D^{25}$ +8.9 (c = 0.97, MeOH), (lit.[3] $[\alpha]_D^{25}$

+3.37 (c = 0.1, MeOH)); υ_{max}/cm^{-1}(neat) 3304 (N-H), 1699 (C=O), 1600 (C=C), 1549 (N-H); δ_H (400 MHz, $CDCl_3$) 5.99 (1H, d, *J* 8.5 Hz, N*H*CH), 5.82 (1H, ddt, *J* 17.0, 10.0, 6.5 Hz, CH_2=C*H*), 5.00 (1H, dq, *J* 17.0, 1.5 Hz, CH_2=CH), 4.94 (1H, ddt, 10.0, 2.0, 1.0 CH_2=CH), 4.62 (1H, dd, *J* 8.5, 5.0 Hz, NHC*H*), 2.29 - 2.24 (2H, m, CH_2CONH), 2.08 - 2.01 (2H, m, CH_2=CHCH_2), 2.02 - 1.92 (1H, m, C*H*CH_3), 1.65 (2H, quin., *J* 7.5 Hz, CH_2CH_2CO), 1.52 (1H, dqd, *J* 15.0, 7.5, 4.5 Hz, CHCH_2CH_3), 1.41 - 1.34 (11H, m, CHCH_2CH_3, 5 x CH_2), 0.97 (3H, d, *J* 7.0 Hz, CHCH_3), 0.95 (3H, t, *J* 7.5 Hz, CH_2CH_3); δ_H (700 MHz, DMSO-d_6) 12.44 (1H, br. s., CO_2*H*), 7.88 (1H, d, *J* 8.3 Hz, N*H*CH), 5.79 (1H, ddt *J* 17.0, 10.1, 6.6 Hz, CH_2=C*H*), 5.02 - 4.97 (1H, m, CH_2=CH), 4.95 - 4.91 (1H, m, CH_2=CH), 4.17 (1H, dd, *J* 7.9, 6.9 Hz, NHC*H*), 2.27 – 2.20 (2H, m, CH_2CONH), 2.00 (2H, q, *J* 6.9 Hz, CH_2=CHCH_2), 1.79 - 1.71 (1H, m, C*H*CH_3), 1.51 - 1.43 (2H, m, CH_2CH_2CO), 1.43 - 1.36 (1H, m, CHCH_2CH_3), 1.36 - 1.31 (2H, m, CH_2), 1.28 - 1.21 (8H, m, 4 x CH_2), 1.21 - 1.14 (1H, m, CHCH_2CH_3), 0.86 - 0.82 (6H, m, CHCH_3, $CHCH_2$CH_3); δ_H (700 MHz, CD_3OD) 5.80 (1H, ddt, *J* 17.0, 10.2, 6.8 Hz, CH_2=C*H*), 4.97 (1H, dq, *J* 17.1, 2.1 Hz, CH_2=CH), 4.91 (1H, dt, *J* 10.3, 1.0 Hz, CH_2=CH), 4.37 (1H, d, *J* 5.8 Hz, NHC*H*), 2.29 - 2.22 (2H, m CH_2CONH), 2.07 - 2.02 (2H, m, CH_2=CHCH_2), 1.92- 1.84 (1H, m, C*H*CH_3), 1.64 - 1.58 (2H, m, CH_2CH_2CO), 1.52 (1H, dqd, *J* 15.0, 7.5, 4.4 Hz, CHCH_2CH_3), 1.42 - 1.37 (2H, m, CH_2), 1.35 - 1.29 (8H, m, 4 x CH_2), 1.27 - 1.23 (1H, m, CHCH_2CH_3), 0.95 (3H, d, *J* 6.7 Hz, CHCH_3), 0.93 (3H, t, *J* 7.4 Hz, CH_2CH_3); δ_C (100 MHz, $CDCl_3$) 175.6, 174.0 (*C*O_2H, *C*ONH), 139.2 (CH_2=*C*H), 114.2 (*C*H_2=CH), 56.4 (*C*HNH), 37.7 (*C*HCH_3), 36.7 (*C*H_2CONH), 33.8 (CH_2=CH*C*H_2), 29.3, 29.2, 29.1, 28.9 (5 x *C*H_2), 25.7 (*C*H_2CH_2CO), 25.1 (CH*C*H_2CH_3), 15.4 (CH*C*H_3), 11.6 ($CHCH_2$*C*H_3); *m/z* (ESI) 296.2 ([M-H]-, 100%); HR-ESIMS: calculated for $C_{17}H_{31}NO_3Na$: 320.2196, found 320.1297 $[M+Na]^+$. Data are consistent with that previously reported.[3]

(3*S*, 2*S*)-2-(Undec-10'-enoylamino)-3-methyl-pentanoic acid and (2*R*, 3*S*)-2-(undec-10'-enoylamino)-3-methyl-pentanoic acid 3.03

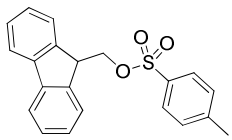


Method modified from literature procedure by *du* Vigneaud and Meyer.[4] (2*S,* 3*S*)-2-(Undec-10'-enoylamino)-3-methyl-pentanoic acid **3.02** (2.0 g, 6.7 mmol) was dissolved in 3M aqueous sodium hydroxide (2.3 mL, 6.7 mmol), and THF (4 mL) and water (4 mL) were added. Acetic anhydride (0.62 mL, 6.7 mmol) was added and the reaction mixture was heated to 65 °C for 3 days. The mixture was concentrated *in vacuo* and partitioned between pH 2 buffer (10 mL) and EtOAc (10 mL). The aqueous phase was further extracted with EtOAc (2 x 10 mL). The combined organic extracts were washed with water (10 mL) and saturated aqueous NaCl solution (10 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford the mix of diastereomeric products **3.03** as a white solid (1.51 g, 5.1 mmol, 76 %, 82: 18 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR); υ_{max}/cm^{-1} (neat) 3303 (N-H), 1697 (C=O), 1597 (C=C), 1549 (N-H); δ_H (400 MHz, $CDCl_3$) 7.98 ((2*S,* 3*S*), 1H, s, CO_2H; (2*R,* 3*S*), 1H, s, CO_2H), 6.16 ((2*R,* 3*S*), 1H, d, *J* 8.0 Hz, CHN*H*), 6.07 ((2*S,* 3*S*), 1H, d, *J* 8.0 Hz, CHN*H*), 5.81 ((2*S,* 3*S*), 1H, ddt, *J* 17.0, 10.0, 6.5 Hz, CH_2=C*H*; (2*R,* 3*S*), 1H, ddt, *J* 17.0, 10.0, 6.5 Hz, CH_2=C*H*), 4.99 ((2*S,* 3*S*), 1H, m, CH_2=CH; (2*R,* 3*S*), 1H, m, CH_2=CH), 4.93 ((2*S,* 3*S*), 1H, m, CH_2=CH; (2*R,* 3*S*), 1H, m, CH_2=CH), 4.75 ((2*R,* 3*S*), 1H, dd, *J* 9.0, 4.0 Hz, C*H*NH), 4.63 ((2*S,* 3*S*), 1H, dd, *J* 8.5, 5.0 Hz, C*H*NH), 2.26 ((2*S,* 3*S*), 2H, td, *J* 7.5, 4.0 Hz, CH_2CONH; (2*R,* 3*S*), 2H, td, *J* 7.5, 4.0 Hz, CH_2CONH), 2.03 ((2*S,* 3*S*), 2H, q, *J* 6.5 Hz, CH_2=CHCH_2; (2*R,* 3*S*), 2H, q, *J* 6.5 Hz, CH_2=CHCH_2), 1.99 - 1.86 ((2*S,* 3*S*), 1H, m, C*H*CH_3; (2*R,* 3*S*), 1H, m, C*H*CH_3), 1.68 - 1.59 ((2*S,* 3*S*), 2H, m, CH_2CH_2CONH; (2*R,* 3*S*), 2H, m, CH_2CH_2CONH), 1.55 - 1.13 ((2*S,* 3*S*), 12H, m, CHCH_2CH_3, 5 x CH_2; (2*R,* 3*S*), 12H,

m, CHC*H*$_2$CH$_3$, 5 x C*H*$_2$), 1.00 - 0.88 ((2*S,* 3*S*), 6H, m, CHCH$_2$C*H*$_3$, CHC*H*$_3$; (2*R,* 3*S*), 6H, m, CHCH$_2$C*H*$_3$, CHC*H*$_3$); δ_H (700 MHz, DMSO-d$_6$) 12.43 ((2*S,* 3*S*), 1H, br. s., CO$_2$*H*; (2*R,* 3*S*), 1H, br. s., CO$_2$*H*), 7.88 ((2*S,* 3*S*), 1H, d, *J* 8.3 Hz, N*H*CH), 7.79 ((2*R,* 3*S*), 1H, d, *J* 8.3 Hz, N*H*CH), 5.79 ((2*S,* 3*S*), 1H, ddt, *J* 16.9, 10.1, 6.8 Hz, CH$_2$=C*H*; (2*R,* 3*S*), 1H, ddt, *J J* 16.9, 10.1, 6.8 Hz, CH$_2$=C*H*), 4.99 ((2*S,* 3*S*), 1H, d, *J* 17.0 Hz, C*H*$_2$=CH; (2*R,* 3*S*), 1H, d, *J* 17.0 Hz, C*H*$_2$=CH), 4.93 ((2*S,* 3*S*), 1H, d, *J* 10.3 Hz, C*H*$_2$=CH; (2*R,* 3*S*), 1H, d, *J* 10.3 Hz, C*H*$_2$=CH), 4.35 ((2*R,* 3*S*), 1H, dd, *J* 8.5, 5.0 Hz, NHC*H*), 4.17 ((2*S,* 3*S*), 1H, dd, *J* 8.0, 6.4 Hz, NHC*H*), 2.20 - 2.08 ((2*S,* 3*S*), 2H, m, C*H*$_2$CONH; (2*R,* 3*S*), 2H, m, C*H*$_2$CONH), 2.00 ((2*S,* 3*S*), 2H, q, *J* 6.8 Hz, CH$_2$=CHC*H*$_2$; (2*R,* 3*S*), 2H, q, *J* 6.8 Hz, CH$_2$=CHC*H*$_2$), 1.88 - 1.81 ((2*R,* 3*S*), 1H, m, C*H*CH$_3$), 1.78 - 1.72 ((2*S,* 3*S*), 1H, m, C*H*CH$_3$), 1.51 - 1.43 ((2*S,* 3*S*), 2H, m, C*H*$_2$; (2*R,* 3*S*), 2H, m, C*H*$_2$), 1.42 - 1.37 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, CHC*H*$_2$CH$_3$), 1.36 - 1.31 ((2*S,* 3*S*), 2H, m, C*H*$_2$; (2*R,* 3*S*), 2H, m, C*H*$_2$), 1.28 - 1.21 ((2*S,* 3*S*), 8H, m, 4 x C*H*$_2$; (2*R,* 3*S*), 8H, m, 4 x C*H*$_2$), 1.21 - 1.12 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, CHC*H*$_2$CH$_3$), 0.86 - 0.83 ((2*S,* 3*S*), 6H, m, CHC*H*$_3$, CHCH$_2$C*H*$_3$; (2*R,* 3*S*), 6H, m, CHC*H*$_3$, CHCH$_2$C*H*$_3$); δ_H (700 MHz, CD$_3$OD) 5.80 ((2*S,* 3*S*), 1H, ddt, *J* 17.0, 10.2, 6.8 Hz, CH$_2$=C*H*; (2*R,* 3*S*), 1H, ddt, *J* 17.0, 10.2, 6.8 Hz, CH$_2$=C*H*), 4.98 ((2*S,* 3*S*), 1H, dq, *J* 17.1, 1.6 Hz, C*H*$_2$=CH; (2*R,* 3*S*), 1H, dq, *J* 17.1, 1.6 Hz, C*H*$_2$=CH), 4.91 ((2*S,* 3*S*), 1H, dt, *J* 10.1, 1.0 Hz, C*H*$_2$=CH; (2*R,* 3*S*), 1H, dt, *J* 10.1, 1.0 Hz, C*H*$_2$=CH), 4.54 ((2*R,* 3*S*), 1H, d, *J* 4.7 Hz, NHC*H*) 4.37 ((2*S,* 3*S*), 1H, d, *J* 5.8 Hz, NHC*H*), 2.31 - 2.22 ((2*S,* 3*S*), 2H, m, C*H*$_2$CONH; (2*R,* 3*S*), 2H, m, C*H*$_2$CONH), 2.07 - 2.02 ((2*S,* 3*S*), 2H, m, CH$_2$=CHC*H*$_2$; (2*R,* 3*S*), 2H, m, CH$_2$=CHC*H*$_2$), 1.99 - 1.95 ((2*R,* 3*S*), 1H, m, C*H*CH$_3$), 1.92 - 1.85 ((2*S,* 3*S*), 1H, m, C*H*CH$_3$), 1.61 ((2*S,* 3*S*), 2H, quin, *J* 7.0 Hz, C*H*$_2$CH$_2$CONH; (2*R,* 3*S*), 2H, quin, *J* 7.0 Hz, C*H*$_2$CH$_2$CONH), 1.52 ((2*S,* 3*S*), 1H, dqd, *J* 15.0, 7.5, 4.2 Hz, CHC*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, dqd, *J* 15.0, 7.5, 4.2 Hz, CHC*H*$_2$CH$_3$), 1.42 - 1.36 ((2*S,* 3*S*), 2H, m, C*H*$_2$; (2*R,* 3*S*), 2H, m, C*H*$_2$), 1.32 ((2*S,* 3*S*), 8H, m, 4 x C*H*$_2$; (2*R,* 3*S*), 8H, m, 4 x C*H*$_2$), 1.28 - 1.21 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, CHC*H*$_2$CH$_3$), 0.97 - 0.92 ((2*S,* 3*S*), 6H, m, CHCH$_2$C*H*$_3$, CHC*H*$_3$; (2*R,* 3*S*), 6H, m, CHCH$_2$C*H*$_3$, CHC*H*$_3$);

δ_C (100 MHz, $CDCl_3$) 175.6, 174.1, 174.0 ((2*S,* 3*S*), *C*O_2H, *C*ONH; (2*R,* 3*S*), *C*O_2H, *C*ONH), 139.2 ((2*S,* 3*S*), CH_2=*C*H; (2*R,* 3*S*), CH_2=*C*H), 114.2 ((2*S,* 3*S*), *C*H_2=CH; (2*R,* 3*S*), *C*H_2=CH), 56.4 ((2*S,* 3*S*), *C*HNH), 55.2 ((2*R,* 3*S*), *C*HNH), 37.7, 37.6 ((2*S,* 3*S*), *C*HCH_3; (2*R,* 3*S*), *C*HCH_3), 36.7 ((2*S,* 3*S*), *C*H_2CONH; (2*R,* 3*S*), *C*H_2CONH), 33.8 ((2*S,* 3*S*), CH_2=CH*C*H_2; (2*R,* 3*S*), CH_2=CH*C*H_2), 29.3, 29.3, 29.2, 29.1, 28.9 ((2*S,* 3*S*), 5 x *C*H_2; (2*R,* 3*S*), 5 x *C*H_2), 25.7 ((2*S,* 3*S*), *C*H_2CH_2CO; (2*R,* 3*S*), *C*H_2CH_2CO), 25.1 ((2*S,* 3*S*), CH*C*H_2CH_3; (2*R,* 3*S*), CH*C*H_2CH_3), 15.4, 14.6 ((2*S,* 3*S*), CH*C*H_3; (2*R,* 3*S*), CH*C*H_3), 11.6 ((2*S,* 3*S*), $CHCH_2$*C*H_3; (2*R,* 3*S*), $CHCH_2$*C*H_3); *m/z* (ESI-) 296.1 ($[M\text{-}H^+]^-$, 100%); HR-ESIMS: calculated for $C_{17}H_{32}NO_3$: 298.2377, found 298.2383 $[M+H]^+$.

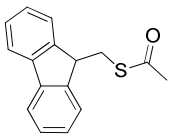
9-Fluorenylmethyl *p*-toluenesulfonate 3.06



Method modified from literature procedure by Albericio *et al.*[5] To a solution of 9-fluorenemethanol (4.00 g, 20.4 mmol) in chloroform (20 mL) at 0°C was added dropwise a solution of toluenesulfonyl chloride (4.80 g, 24.5 mmol) and pyridine (3.2 mL, 40.8 mmol) in chloroform (10 mL). The reaction mixture was allowed to reach room temperature overnight to give a bright yellow solution. pH 2 buffer (20 mL) was added and the organic extract was further washed with pH 2 buffer (20 mL), water (20 mL) saturated aqueous $NaHCO_3$ solution (2 x 20 mL) and saturated aqueous NaCl solution (20 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the crude product as a yellow solid. Recrystallisation from $CHCl_3$:hexanes afforded **3.06** as glossy white crystals (3.95 g, 11.3 mmol, 55 %); m.p. 111 - 112 °C (lit.[6] 115 °C); υ_{max}/cm^{-1} (neat) 1356 (S=O); δ_H (400 MHz, $CDCl_3$) 7.77 (2H, *J* 8.5 Hz, fluorenylmethyl 2 x Ar C*H*), 7.74 (2H, d, *J* 7.5 Hz, fluorenylmethyl 2 x Ar C*H*), 7.55 (2H, d, *J* 7.5 Hz, 2 x tosyl Ar C*H*), 7.40 (2H, t, *J* 7.5 Hz, fluorenylmethyl 2 x Ar C*H*), 7.31 (2H, d, *J* 8.5 Hz, 2 x tosyl Ar C*H*), 7.30 (2H, dd, *J* 7.5, 1.0 Hz, fluorenylmethyl 2 x Ar C*H*), 4.30 - 4.21 (3H, m, C*H*CH_2, CHCH_2), 2.43 (3H, s,

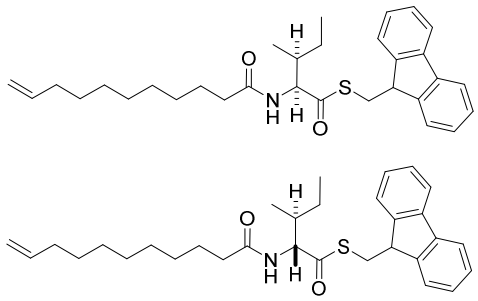
C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 144.9, 142.5 (2 x fluorenylmethyl Ar *C*), 141.2, 132.8, (2 x tosyl Ar *C*), 129.9, 128.1 (2 x tosyl Ar *CH*), 127.9, 127.2, 125.2, 120.1 (4 x fluorenylmethyl Ar *CH*), 71.9 (CH*C*H$_2$), 46.7 (*C*HCH$_2$), 21.6 (*C*H$_3$); *m/z* (ESI+) 373.0 ([M+Na], 100%); HR-ESIMS: calculated for $C_{21}H_{18}O_3SNa$: 373.0867, found 373.0869 $[M+Na]^+$. Data are consistent with that previously reported.[5]

9-Fluorenylmethyl thioacetate 3.07



Method modified from literature procedure by Crich *et al.*[1] A solution of potassium thioacetate (4.26 g, 37.3 mmol) and 9-fluorenylmethyl *p*-toluenesulfonate **3.06** (6.49 g, 18.7 mmol) in DMF (65 mL) was stirred at room temperature for 2 hours to give a bright orange solution. EtOAc (150 mL) and water (150 mL) were added and the phases separated. The organic phase was washed with water (150 mL) and saturated aqueous NaCl solution (150 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give a bright orange oil. Purification by silica chromatography (4 % EtOAc: hexane) gave the thioacetate **3.07** as a cream coloured crystalline solid (3.24 g, 12.7 mmol, 68%); m.p. 73 – 74 °C (lit.[1] 72 – 73 °C); υ_{max} /cm^{-1} (neat) 1681 (C=O); δ_H (400 MHz, $CDCl_3$) 7.77 (2H, d, *J* 8.0 Hz, 2 x Ar C*H*), 7.67 (2H, d, *J* 7.5 Hz, 2 x Ar C*H*), 7.41 (2H, t, *J* 7.5 Hz, 2 x Ar C*H*), 7.34 (2H, t, *J* 7.5 Hz, 2 x Ar C*H*), 4.19 (1H, t, *J* 6.0 Hz, C*H*CH$_2$), 3.55 (2H, d, *J* 6.0 Hz, CHC*H*$_2$), 2.29 (3H, s, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 195.4 (S*C*OCH$_3$), 145.4, 141.0 (4 x Ar *C*), 127.7, 127.1, 124.6, 119.9 (4 x Ar *C*H), 46.6 (*C*HCH$_2$), 32.4 (CH*C*H$_2$), 30.6 (CH$_3$); *m/z* (ESI+) 211.0 ([M-SOCH$_3$)], 100%); HR-ESIMS: calculated for $C_{16}H_{15}OS$: 255.0838, found 255.0837 $[M+H]^+$. Data are consistent with that previously reported.[1]

(2*S*, 3*S*) and (2*R*, 3*S*)-(9'-Fluorenylmethyl)-2-(undec-10-enoylamino)-3-methyl pentanethioate 3.04



Method modified from literature procedure by Crich *et al.*[1] A solution of thioacetate **3.07** (2.40 g, 9.44 mmol) in dry Et_2O (80 mL) was cooled to -78°C under N_2. A solution of DIBAL (25% w/w in toluene, 14 mL, 20.8 mmol) was added dropwise and the reaction mixture held at -78°C for 5 minutes. The reaction mixture was allowed to warm to 0°C over 1 hour and quenched with EtOAc (30 mL, 102 mmol). An aqueous solution of Rochelle's salt (1.4M, 120 mL) was added and the mixture was stirred vigorously for 1 hour to give a biphasic solution. The aqueous phase was extracted with Et_2O (2 x 40 mL) and the combined organics washed with pH 2 buffer (20 mL), water (20 mL), saturated aqueous NaCl solution (20 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the crude thiol as a yellow oil, which was purified by silica chromatography (2 % EtOAc:hexanes) to give the thiol as a yellow oil, which was used immediately (1.41 g, 6.64 mmol, 70%); υ_{max} /cm^{-1} (neat) 2565 (S-H);

A solution of 9-fluorenylmethylthiol (0.93 g, 4.4 mmol) and acid **3.02** (1.00 g, 3.36 mmol) in DMF (55 mL) was stirred at -20°C for 20 minutes. PyBOP (4.37 g, 8.41 mmol) and diisopropylethylamine (1.40 mL, 8.41 mmol) were added and the reaction mixture was stirred at -20 °C for 4 hours. The reaction mixture was quenched with pH 2 buffer (30 mL) and extracted with CH_2Cl_2 (2 x 20 mL). The combined organic extracts were concentrated *in vacuo* and the residue was partitioned between water (20 mL) and EtOAc (20 mL). The separated organic phase was washed with pH 2 buffer (10 mL), water (10 mL), saturated aqueous $NaHCO_3$

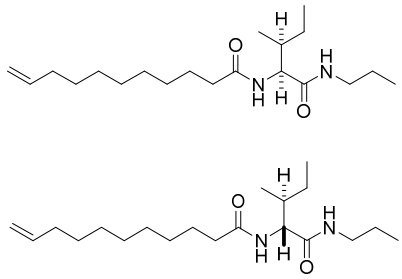
solution (10 mL) and saturated aqueous NaCl solution (10 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the crude thioester (61: 39 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomer before purification, calculated by ^{1}H NMR). The thioester **3.04** was obtained as by silica column chromatography (100 % pet. ether to 90 % pet.ether: EtOAc) as a yellow waxy solid (0.56 g, 1.14 mmol, 34 %, 52: 48 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR); υ_{max}/cm^{-1} (neat) 3283 (N-H), 1686 (thioester C=O), 1635 (amide C=O), 1531 (N-H); δ_H (400 MHz, $CDCl_3$) 7.73 ((2*S,* 3*S*), 2H, d, *J* 7.5 Hz, 2 x Ar C*H*; (2*R,* 3*S*), 2H, d, *J* 7.5 Hz, 2 x Ar C*H*), 7.63 - 7.59 ((2*S,* 3*S*), 2H, m, 2 x Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x Ar C*H*), 7.37 ((2*S,* 3*S*), 2H, t, *J* 7.5 Hz, 2 x Ar C*H*; (2*R,* 3*S*), 2H, t, *J* 7.5 Hz, 2 x Ar C*H*), 7.32 - 7.27 ((2*S,* 3*S*), 2H, m, 2 x Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x Ar C*H*), 5.80 ((2*S,* 3*S*), 1H, ddt, *J* 17.0, 10.0, 6.5 Hz, CH_2=C*H*; (2*R,* 3*S*), 1H, ddt, *J* 17.0, 10.0, 6.5 Hz, CH_2=C*H*), 5.73 ((2*S,* 3*S*), 1H, t, *J* 9.0 Hz, N*H*CH; (2*R,* 3*S*), 1H, t, *J* 9.0 Hz, N*H*CH), 5.02 - 4.90 ((2*S,* 3*S*), 2H, m, C*H*$_2$=CH; (2*R,* 3*S*), 2H, m, C*H*$_2$=CH), 4.74 ((2*R,* 3*S*), 1H, dd, *J* 9.5, 4.0 Hz, NHC*H*), 4.61 ((2*S,* 3*S*), 1H, dd, *J* 9.0, 5.0 Hz, NHC*H*), 4.17 ((2*S,* 3*S*), 1H, t, *J* 5.5 Hz, C*H*CH_2S; (2*R,* 3*S*), 1H, t, *J* 5.5 Hz, C*H*CH_2S), 3.63 - 3.52 ((2*S,* 3*S*), 2H, m, CHC*H*$_2$S; (2*R,* 3*S*), 2H, m, CHC*H*$_2$S), 2.20 ((2*S,* 3*S*), 2H, q, *J* 7.0 Hz, C*H*$_2$CONH; (2*R,* 3*S*), 2H, q, *J* 7.0 Hz, C*H*$_2$CONH), 2.03 ((2*S,* 3*S*), 2H, q, *J* 7.0 Hz, CH=CH_2C*H*$_2$; (2*R,* 3*S*), 2H, q, *J* 7.0 Hz, CH=CH_2C*H*$_2$), 1.93 - 1.77 ((2*S,* 3*S*), 1H, m, C*H*CH_3; (2*R,* 3*S*), 1H, m, C*H*CH_3), 1.67 - 1.56 ((2*S,* 3*S*), 2H, m, C*H*$_2$$CH_2$CONH; (2*R,* 3*S*), 2H, m, C*H*$_2$$CH_2$CONH), 1.40 - 1.01 ((2*S,* 3*S*), 12H, m, CHC*H*$_2$$CH_3$, 5 x C*H*$_2$; (2*R,* 3*S*), 12H, m, CHC*H*$_2$$CH_3$, 5 x C*H*$_2$), 0.81 - 0.60 ((2*S,* 3*S*), 6H, m, $CHCH_2$C*H*$_3$, CHC*H*$_3$; (2*S,* 3*S*), 6H, m, $CHCH_2$C*H*$_3$, CHC*H*$_3$); δ_H (700 MHz, DMSO-d_6) 8.07 ((2*R,* 3*S*), 1H, d, *J* 8.0 Hz, N*H*CH), 8.01 ((2*S,* 3*S*), 1H, d, *J* 8.7 Hz, N*H*CH), 7.84 ((2*S,* 3*S*), 2H, t, *J* 6.3 Hz, 2 x Ar C*H*; (2*R,* 3*S*), 2H, t, *J* 6.3 Hz, 2 x Ar C*H*), 7.68 - 7.56 ((2*S,* 3*S*), 2H, m, 2 x Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x Ar C*H*), 7.38 ((2*S,* 3*S*), 2H, dd, *J* 12.2, 5.8 Hz, 2 x Ar C*H*; (2*R,* 3*S*), 2H, dd, *J* 12.2, 5.8 Hz, 2 x Ar C*H*), 7.34 - 7.26 ((2*S,* 3*S*), 2H, m, 2 x Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x Ar C*H*), 5.78 ((2*S,* 3*S*), 1H, ddt, *J* 16.7, 10.3, 6.7 Hz, CH_2=C*H*; (2*R,* 3*S*), 1H, ddt, *J* 16.7, 10.3, 6.7 Hz, CH_2=C*H*), 4.98 ((2*S,* 3*S*), 1H, d, *J*

17.0 Hz, C*H*$_2$=CH; (2*R,* 3*S*), 1H, d, *J* 17.0 Hz, C*H*$_2$=CH), 4.93 ((2*S,* 3*S*), 1H, d, *J* 10.3 Hz, C*H*$_2$=CH; (2*R,* 3*S*), 1H, d, *J* 10.3 Hz, C*H*$_2$=CH), 4.33 ((2*R,* 3*S*), 1H, dd, *J* 8.7, 5.1 Hz, NHC*H*), 4.29 - 4.25 ((2*S,* 3*S*), 1H, m, C*H*CH$_2$S; (2*R,* 3*S*), 1H, m, C*H*CH$_2$S), 4.15 ((2*S,* 3*S*), 1H, t, *J* 7.4 Hz, NHC*H*), 3.72 - 3.65 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$S; (2*R,* 3*S*), 1H, m, CHC*H*$_2$S), 3.58 ((2*S,* 3*S*), 1H, *J* 13.6, 5.0 Hz, CHC*H*$_2$S; (2*R,* 3*S*), 1H, *J* 13.6, 5.0 Hz, CHC*H*$_2$S), 2.13 - 2.06 ((2*S,* 3*S*), 2H, m, NHCOC*H*$_2$; (2*R,* 3*S*), 2H, m, NHCOC*H*$_2$), 1.99 ((2*S,* 3*S*), 2H, q, *J* 6.9 Hz, CH$_2$=CHC*H*$_2$; (2*R,* 3*S*), 2H, q, *J* 6.9 Hz, CH$_2$=CHC*H*$_2$), 1.75 - 1.67, 1.64- 1.58 (2*S,* 3*S*), 1H, m, C*H*CH$_3$; (2*R,* 3*S*), 1H, m, C*H*CH$_3$), 1.46 - 1.39 ((2*S,* 3*S*), 2H, m, C*H*$_2$; (2*R,* 3*S*), 2H, m, C*H*$_2$), 1.37 - 1.29 ((2*S,* 3*S*), 2H, m, C*H*$_2$; (2*R,* 3*S*), 2H, m, C*H*$_2$), 1.26 - 1.15 ((2*S,* 3*S*), 8H, m, 4 x C*H*$_2$; (2*R,* 3*S*), 8H, m, 4 x C*H*$_2$), 1.09 - 1.01 ((2*S,* 3*S*), 1H, m, C*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, C*H*$_2$CH$_3$), 1.01 - 0.89 ((2*S,* 3*S*), 1H, m, C*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, C*H*$_2$CH$_3$), 0.71 ((2*R,* 3*S*), 3H, t, *J* 7.4 Hz, CHCH$_2$C*H*$_3$), 0.67 ((2*S,* 3*S*), 3H, t, *J* 7.4 Hz, CHCH$_2$C*H*$_3$), 0.59 ((2*S,* 3*S*), 3H, *J* 6.7 Hz, CHC*H*$_3$), 0.55 ((2*R,* 3*S*), 3H, *J* 6.7 Hz, CHC*H*$_3$); δ_H (700 MHz, CD$_3$OD) 7.75 ((2*S,* 3*S*), 2H, d, *J* 7.5 Hz, 2 x Ar C*H*; (2*R,* 3*S*), 2H, d, *J* 7.5 Hz, 2 x Ar C*H*), 7.61 ((2*S,* 3*S*), 2H, dd, *J* 12.3, 7.6 Hz, 2 x Ar C*H*; (2*R,* 3*S*), 2H, dd, *J* 12.3, 7.6 Hz, 2 x Ar C*H*), 7.36 ((2*S,* 3*S*), 2H, t, *J* 7.5 Hz, 2 x Ar C*H*; (2*R,* 3*S*), 2H, t, *J* 7.5 Hz, 2 x Ar C*H*), 7.30 - 7.26 ((2*S,* 3*S*), 2H, m, 2 x Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x Ar C*H*), 5.84 – 5.77 ((2*S,* 3*S*), 1H, m, CH$_2$=C*H*; (2*R,* 3*S*), 1H, m, CH$_2$=C*H*), 4.99 - 4.94 ((2*S,* 3*S*), 1H, m, C*H*$_2$=CH; (2*R,* 3*S*), 1H, m, C*H*$_2$=CH), 4.93 - 4.90 ((2*S,* 3*S*), 1H, m, C*H*$_2$=CH; (2*R,* 3*S*), 1H, m, C*H*$_2$=CH), 4.48 ((2*R,* 3*S*), 1H, d, *J* 5.3 Hz, NHC*H*), 4.31 ((2*S,* 3*S*), 1H, d, *J* 6.1 Hz, NHC*H*), 4.23 - 4.20 ((2*S,* 3*S*), 1H, m, C*H*CH$_2$S; (2*R,* 3*S*), 1H, m, C*H*CH$_2$S), 3.73 - 3.68 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$S; (2*R,* 3*S*), 1H, m, CHC*H*$_2$S), 3.63 - 3.58 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$S; (2*R,* 3*S*), 1H, m, CHC*H*$_2$S), 2.30 - 2.16 ((2*S,* 3*S*), 2H, m, NHCOC*H*$_2$; (2*R,* 3*S*), 2H, m, NHCOC*H*$_2$), 2.05 - 2.02 ((2*S,* 3*S*), 2H, m, CH=CH$_2$C*H*$_2$; (2*S,* 3*S*), 2H, m, CH=CH$_2$C*H*$_2$), 1.87 - 1.80, 1.78 - 1.72 ((2*S,* 3*S*), 1H, m, C*H*CH$_3$; (2*R,* 3*S*), 1H, m, C*H*CH$_3$), 1.59 - 1.49 ((2*S,* 3*S*), 2H, m, C*H*$_2$; (2*R,* 3*S*), 2H, m, C*H*$_2$), 1.40 - 1.34 ((2*S,* 3*S*), 2H, m, C*H*$_2$; (2*R,* 3*S*), 2H, m, C*H*$_2$), 1.33 - 1.25 ((2*S,* 3*S*), 8H, m, 4 x C*H*$_2$; (2*R,*

3*S*), 8H, m, 4 x C*H*$_2$), 1.21 - 1.10 ((2*S,* 3*S*), 1H, m, C*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, C*H*$_2$CH$_3$), 1.09 - 0.97 ((2*S,* 3*S*), 1H, m, C*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, C*H*$_2$CH$_3$), 0.81 ((2*R,* 3*S*), 3H, t, *J* 7.4 Hz, CHCH$_2$C*H*$_3$), 0.77 ((2*S,* 3*S*), 3H, t, *J* 7.4 Hz, CHCH$_2$C*H*$_3$), 0.71 ((2*S,* 3*S*), 3H, d, *J* 6.7 Hz, CHC*H*$_3$), 0.63 ((2*S,* 3*S*), 3H, d, *J* 6.9 Hz, CHC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 200.3, 199.7 (S*C*OCH$_3$), 173.1, 173.0 (NH*C*OCH$_2$), 145.2, 145.2, 141.2 ((2*S,* 3*S*), 4 x Ar *C*; (2*R,* 3*S*), 4 x Ar *C*), 139.2 ((2*S,* 3*S*), CH$_2$=*C*H; (2*R,* 3*S*), CH$_2$=*C*H), 127.7, 127.1, 124.7, 119.9 ((2*S,* 3*S*), 8 x Ar *C*H; (2*R,* 3*S*), 8 x Ar *C*H), 114.2 ((2*S,* 3*S*), *C*H$_2$=CH; (2*R,* 3*S*), *C*H$_2$=CH), 63.2 ((2*S,* 3*S*), NH*C*H), 61.8 ((2*R,* 3*S*), NH*C*H), 46.7 ((2*S,* 3*S*), *C*HCH$_2$S; (2*R,* 3*S*), *C*HCH$_2$S), 37.8 ((2*S,* 3*S*), CH$_2$*C*HCH$_3$; (2*R,* 3*S*), CH$_2$*C*HCH$_3$), 36.7 ((2*S,* 3*S*), NHCO*C*H$_2$; (2*R,* 3*S*), NHCO*C*H$_2$), 33.8 ((2*S,* 3*S*), CH$_2$=CH*C*H$_2$; (2*R,* 3*S*), CH$_2$=CH*C*H$_2$), 31.9, 31.8 ((2*S,* 3*S*), S*C*H$_2$; (2*R,* 3*S*), S*C*H$_2$), 29.3, 29.3, 29.2, 29.1, 28.9 ((2*S,* 3*S*), 5 x *C*H$_2$; (2*R,* 3*S*), 5 x *C*H$_2$), 26.5 ((2*S,* 3*S*), NHCOCH$_2$*C*H$_2$; (2*R,* 3*S*), NHCOCH$_2$*C*H$_2$), 25.6 ((2*S,* 3*S*), CH*C*H$_2$CH$_3$; (2*R,* 3*S*), CH*C*H$_2$CH$_3$), 15.5, 14.2, 13.9, 11.7 ((2*S,* 3*S*), CH*C*H$_3$, CH$_2$*C*H$_3$; (2*R,* 3*S*), CH*C*H$_3$, CH$_2$*C*H$_3$); *m/z* (ESI+) 514.3 ([M+Na], 100%), 492.3 ([M+H], 13%); HR-ESIMS: calculated for $C_{31}H_{42}NO_2S$: 492.2931, found 492.2951 $[M+H]^+$.

This compound was also prepared by the same method using a mix of L- and D-*allo*isoleucine (0.22 g, 0.72 mmol) to give **3.05** as an orange waxy solid (0.134 g, 0.27 mmol, 38%. 35: 65 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR before purification; 60: 40 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR of product after silica column chromatography). All characterisation data was identical to that obtained for **3.04** above.

(2*S*, 3*S*) and (2*R*,3*S*)-(propyl)-2-(undec-10-enoylamino)-3-methyl pentaneamide 3.09



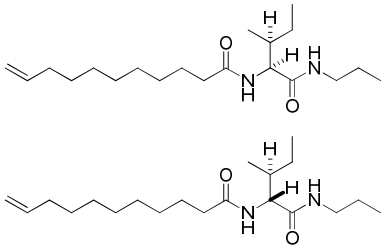
A solution of *n*-propylamine (0.36 mL, 4.37 mmol) and ((2*S,* 3*S*)-2-(undec-10'-enoylamino)-3-methyl-pentanoic acid (1.0 g, 3.36 mmol) in DMF (55 mL) was stirred at -20 °C for 20 minutes. PyBOP (4.37 g, 8.41 mmol) and diisopropylethylamine (1.4 mL, 8.41 mmol) were added and the reaction mixture stirred at -20 °C for 4 hours. The reaction mixture was acidified with pH 2 buffer and extracted with CH_2Cl_2 (2 x 20 mL). The combined organic extracts were concentrated *in vacuo* and the residue was partitioned between water (20 mL) and EtOAc (20 mL). The separated organic phase was washed with pH 2 buffer (10 mL), water (10 mL), saturated aqueous $NaHCO_3$ solution (10 mL) and saturated aqueous NaCl solution (10 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the crude amide **3.09** (86: 14 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomer before purification, calculated by ^{1}H NMR). **3.09** was obtained by silica chromatography (0.81 g, 2.39 mmol, 71 %, 88:12 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR); υ_{max}/cm^{-1} 3282 (N-H), 1631 (C=O), 1539 (N-H); δ_H (400 MHz, $CDCl_3$) 7.30 - 7.15 ((2*S,* 3*S*), 1H, m, CON*H*; (2*R,* 3*S*), 1H, m, CON*H*), 7.00 - 6.82 ((2*S,* 3*S*), 1H, m, CON*H*; (2*R,* 3*S*), 1H, m, CON*H*), 5.77 ((2*S,* 3*S*), 1H, ddt, *J* 17.0, 10.5, 6.5 Hz, CH_2=C*H*; (2*R,* 3*S*), 1H, ddt, *J* 17.0, 10.5, 6.5 Hz, CH_2=C*H*), 4.96 ((2*S,* 3*S*), 1H, dq, *J* 17.0, 2.0 Hz, CH_2=CH; (2*R,* 3*S*), 1H, dq, *J* 17.0, 2.0 Hz, CH_2=CH), 4.95 - 4.90 ((2*S,* 3*S*), 1H, m, CH_2=CH; (2*R,* 3*S*), 1H, m, CH_2=CH), 4.42 ((2*R,* 3*S*), 1H, dd, *J* 9.0, 7.0 Hz, NHC*H*), 4.34 ((2*S,* 3*S*), 1H, t, *J* 9.0 Hz, NHC*H*), 3.33 - 3.19 ((2*S,* 3*S*), 1H, m, NHCH_2; (2*R,* 3*S*), 1H, m, NHCH_2), 3.14 - 3.02 ((2*S,* 3*S*), 1H, m, NHCH_2; (2*R,* 3*S*), 1H, m, NHCH_2), 2.28 - 2.11 ((2*S,* 3*S*), 2H, m,

COCH_2; (2*R,* 3*S*), 2H, m, COCH_2), 2.05 - 1.97 ((2*S,* 3*S*), 2H, m, CH_2=CHCH_2; (2*R,* 3*S*), 2H, m, CH_2=CHCH_2), 1.84 - 1.72 ((2*S,* 3*S*), 1H, m, C*H*CH_3; (2*R,* 3*S*), 1H, m, C*H*CH_3), 1.62 - 1.53 ((2*S,* 3*S*), 5H, m, NHCH_2CH_2, COCH_2CH_2, CHCH_2; (2*R,* 3*S*), 5H, m, NHCH_2CH_2, COCH_2CH_2, CHCH_2), 1.37 - 1.20 ((2*S,* 3*S*), 10H, m, 5 x CH_2; (2*R,* 3*S*), 10H, m, 5 x CH_2), 1.14 - 1.01 ((2*S,* 3*S*), 1H, m, CHCH_2; (2*R,* 3*S*), 1H, m, CHCH_2), 0.92 - 0.81 ((2*S,* 3*S*), 9H, m, CHCH_3, $CHCH_2$CH_3, CH_2CH_3; (2*R,* 3*S*), 9H, m, CHCH_3, $CHCH_2$CH_3, CH_2CH_3); δ_H (700 MHz, DMSO-d_6) 7.88 ((2*S,* 3*S*), 1H, t, *J* 5.3 Hz, N*H*CH_2), 7.81 ((2*R,* 3*S*), 1H, t, *J* 5.0 Hz, N*H*CH_2), 7.74 ((2*S,* 3*S*), 1H, d, *J* 8.7 Hz, N*H*CH), 7.64 ((2*R,* 3*S*), 1H, d, *J* 9.0 Hz, N*H*CH), 5.78 ((2*S,* 3*S*), 1H, ddt, *J* 16.7, 9.6, 6.7 Hz, CH_2=C*H*; (2*R,* 3*S*), 1H, ddt, *J* 16.7, 9.6, 6.7 Hz, CH_2=C*H*), 4.99 ((2*S,* 3*S*), 1H, d, *J* 17.3 Hz, CH_2=CH; (2*R,* 3*S*), 1H, d, *J* 17.3 Hz, CH_2=CH), 4.93 ((2*S,* 3*S*), 1H, d, *J* 10.3 Hz, CH_2=CH; (2*R,* 3*S*), 1H, d, *J* 10.3 Hz, CH_2=CH), 4.25 ((2*R,* 3*S*), 1H, dd, *J* 9.0, 6.1 Hz, NHC*H*), 4.10 ((2*S,* 3*S*), 1H, t, *J* 8.3 Hz, NHC*H*), 3.08 - 3.00 ((2*S,* 3*S*), 1H, m, NHCH_2; (2*R,* 3*S*), 1H, m, NHCH_2), 3.00 - 2.91 ((2*S,* 3*S*), 1H, m, NHCH_2; (2*R,* 3*S*), 1H, m, NHCH_2), 2.22 - 2.04 ((2*S,* 3*S*), 2H, m, NHCOCH_2; (2*R,* 3*S*), 2H, m, NHCOCH_2), 2.00 ((2*S,* 3*S*), 2H, q, *J* 6.9 Hz, CH_2=CHCH_2; (2*R,* 3*S*), 2H, q, *J* 6.9 Hz, CH_2=CHCH_2), 1.78 - 1.71 ((2*R,* 3*S*), 1H, m, C*H*CH_3), 1.71 - 1.64 ((2*S,* 3*S*), 1H, m, C*H*CH_3), 1.52 - 1.43 ((2*S,* 3*S*), 2H, m, CH_2; (2*R,* 3*S*), 2H, m, CH_2), 1.41 - 1.37 ((2*S,* 3*S*), 2H, m, CH_2; (2*R,* 3*S*), 2H, m, CH_2), 1.36 - 1.30 ((2*S,* 3*S*), 2H, m, CH_2; (2*R,* 3*S*), 2H, m, CH_2), 1.27 – 1.19 ((2*S,* 3*S*), 9H, m, 4 x CH_2, CHC$H_2$$CH_3$; (2*R,* 3*S*), 9H, m, 4 x CH_2, CHC$H_2$$CH_3$), 1.12 - 1.01 ((2*S,* 3*S*), 1H, m, CHC$H_2$$CH_3$; (2*R,* 3*S*), 1H, m, CHC$H_2$$CH_3$), 0.85 - 0.77 ((2*S,* 3*S*), 9H, m, CHCH_3, $CHCH_2$CH_3, CH_2CH_2CH_3; (2*R,* 3*S*), 9H, m, CHCH_3, $CHCH_2$CH_3, CH_2CH_2CH_3); δ_H (700 MHz, CD_3OD) 5.80 ((2*S,* 3*S*), 1H, ddt, *J* 17.0, 10.2, 6.7 Hz, CH_2=C*H*; (2*R,* 3*S*), 1H, ddt, *J* 17.0, 10.2, 6.7 Hz, CH_2=C*H*), 4.99 - 4.95 ((2*S,* 3*S*), 1H, m, CH_2=CH; (2*R,* 3*S*), 1H, m, CH_2=CH), 4.92 - 4.89 ((2*S,* 3*S*), 1H, m, CH_2=CH; (2*R,* 3*S*), 1H, m, CH_2=CH), 4.30 ((2*R,* 3*S*), 1H, d, *J* 6.7 Hz, NHC*H*), 4.14 ((2*S,* 3*S*), 1H, d, *J* 8.3 Hz, NHC*H*), 3.19 - 3.09 ((2*S,* 3*S*), 2H, m, NHCH_2; (2*R,* 3*S*), 2H, m, NHCH_2), 2.31 - 2.19 ((2*S,* 3*S*), 2H, m, NHCOCH_2; (2*R,* 3*S*), 2H, m, NHCOCH_2),

2.04 ((2*S,* 3*S*), 2H, q, *J* 7.3 Hz, CH_2=CHC*H*$_2$; (2*R,* 3*S*), 2H, q, *J* 7.3 Hz, CH_2=CHC*H*$_2$), 1.89 - 1.83 ((2*R,* 3*S*), 1H, m, C*H*CH_3), 1.83 - 1.76 ((2*S,* 3*S*), 1H, m, C*H*CH_3), 1.63 - 1.56 ((2*S,* 3*S*), 2H, m, C*H*$_2$; (2*R,* 3*S*), 2H, m, C*H*$_2$), 1.55 - 1.48 (2*S,* 3*S*), 3H, m, C*H*$_2$, CHC*H*$_2$$CH_3$; (2*R,* 3*S*), 3H, m, C*H*$_2$, CHC*H*$_2$$CH_3$), 1.42 - 1.36 ((2*S,* 3*S*), 2H, m, C*H*$_2$; (2*R,* 3*S*), 2H, m, C*H*$_2$), 1.35 – 1.28 ((2*S,* 3*S*), 8H, m, 4 x C*H*$_2$; (2*R,* 3*S*), 8H, m, 4 x C*H*$_2$), 1.21 - 1.13 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$$CH_3$; (2*R,* 3*S*), 1H, m, CHC*H*$_2$$CH_3$), 0.88 - 0.95 ((2*S,* 3*S*), 9H, m, CHC*H*$_3$, $CHCH_2$C*H*$_3$, CH_2CH_2C*H*$_3$; (2*R,* 3*S*), 9H, m, CHC*H*$_3$, $CHCH_2$C*H*$_3$, CH_2CH_2C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 173.2 ((2*S,* 3*S*), CH*C*O; (2*R,* 3*S*), CH*C*O), 171.7 ((2*S,* 3*S*), CH_2*C*O; (2*R,* 3*S*), CH_2*C*O), 139.0 ((2*S,* 3*S*), CH_2=*C*H; (2*R,* 3*S*), CH_2=*C*H), 114.1 ((2*S,* 3*S*), *C*H_2=CH; (2*R,* 3*S*), *C*H_2=CH), 57.5 ((2*S,* 3*S*), *C*HNH), 57.0 (2*R,* 3*S*), *C*HNH), 41.0 ((2*S,* 3*S*), NH*C*H_2; (2*R,* 3*S*), NH*C*H_2), 37.5, 37.1 ((2*S,* 3*S*), *C*HCH_3; (2*R,* 3*S*), *C*HCH_3), 36.4 ((2*S,* 3*S*), CO*C*H_2; (2*R,* 3*S*), CO*C*H_2), 33.7 ((2*S,* 3*S*), CH_2=CH*C*H_2; (2*R,* 3*S*), CH_2=CH*C*H_2), 29.3, 29.2, 29.0, 28.8, 25.93, 25.7 ((2*S,* 3*S*), 5 x *C*H_2, CH*C*$H_2$$CH_3$; (2*R,* 3*S*), 5 x *C*$H_2$, CH*C*$H_2$$CH_3$), 25.1 ((2*S,* 3*S*), $NHCH_2$*C*H_2; (2*R,* 3*S*), $NHCH_2$*C*H_2), 15.3, 14.6 ((2*S,* 3*S*), CH_2CH_2*C*H_3, CH*C*H_3; (2*S,* 3*S*), CH_2CH_2*C*H_3, CH*C*H_3), 11.4, 11.1 ((2*R,* 3*S*), $CHCH_2$*C*H_3; (2*S,* 3*S*), $CHCH_2$*C*H_3); HR-ESIMS: calculated for $C_{20}H_{38}N_2O_2Na$: 361.2825, found 361.2825 $[M+Na]^+$.

This compound was also prepared using the same method using a mix of L- and D-*allo*isoleucine (0.30 g, 1.00 mmol) to give the amide **3.10** as a bright white solid (0.29 g, 0.86 mmol, 86%, 65:35 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR); All characterisation data was identical to that obtained for **3.09** above.

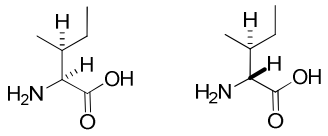
(2*S*, 3*S*) and (2*R*,3*S*)-(propyl)-2-(undec-10-enoylamino)-3-methyl pentaneamide 3.08 from thioester



Method modified from literature procedure by Crich *et al.*[1] A solution of (2*S*, 3*S*) and (2*R*, 3*S*)-(9'-fluorenylmethyl)-2-(undec-10-enoylamino)-3-methyl pentanethioate **3.04** (52: 48 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR; 0.13 g, 0.25 mmol) in 40% piperidine in DMF (2.5 mL) was stirred at room temperature for 1.5 hours. The solution was reduced *in vacuo* and the residue was partitioned between EtOAc (10 mL) and 1M aqueous HCl solution (10 mL). The separated organic phase was washed with water (10 mL) and saturated aqueous NaCl solution (10 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the crude thioacid, which was used immediately without further purification.

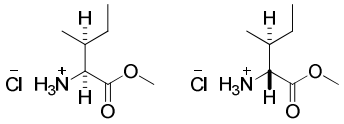
Cs_2CO_3 (0.11 g, 0.32 mmol) was added to a solution of the thioacid (0.25 mmol) in DMF (0.5 mL) under a nitrogen atmosphere. After 10 minutes, a solution of the sulfonamide (0.06 g, 0.21 mmol) in DMF (0.4 mL) was added to give a dark red solution. The reaction mixture was stirred at room temperature for 1 hour, then poured into EtOAc (30 mL). The organics were washed with ice-cold 1M aqueous NaOH solution (10 mL), water (10 mL) and saturated aqueous NaCl solution (10 mL), dried over Na_2SO_4 and concentrated *in vacuo* (86: 14 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomer before purification, calculated by ^{1}H NMR). Purification by silica chromatography (25 % - 50 % EtOAc: pet. ether) gave **3.08** as a yellow oil (29 mg, 0.09 mmol, 35 %, 45:55 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR). All characterisation data was identical to the products **3.09** and **3.10** prepared previously.

L-Isoleucine and D-*allo*-isoleucine 3.11



Method modified from literature procedure by Yamada *et al.*[7] A suspension of L-isoleucine (10.0 g, 76.2 mmol) and acetaldehyde (1.30 mL, 22.9 mmol) in acetic acid (50 mL) was heated to 100 °C for 3 hours to give a dark red solution. The reaction mixture was cooled to room temperature and poured into petroleum ether (300 mL). The mixture was stirred vigorously until a dense brown precipitate formed (*ca.* 1 hour) and the reaction was filtered. The filter cake was triturated with boiling MeOH, filtered and dried *in vacuo* to give a light brown crystalline solid (6.52 g, 49.7 mmol, 65%, 76:24 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR); υ_{max}/cm^{-1}(neat) 3010 (N-H), 2577 (O-H), 1588 (C=O); δ_H (400 MHz, D_2O) 3.71 ((2*R,* 3*S*), 1H, d, *J* 3.5 Hz, NH_2CH), 3.64 ((2*S,* 3*S*), 1H, d, *J* 4.0 Hz, NH_2CH), 2.10 - 1.88 ((2*S,* 3*S*), 1H, m, $CHCH_3$; (2*R,* 3*S*), 1H, m, $CHCH_3$), 1.51 - 1.16 ((2*S,* 3*S*), 2H, m, CH_2CH_3; (2*R,* 3*S*), 2H, m, CH_2CH_3), 1.03 - 0.86 ((2*S,* 3*S*), 6H, m, 2 x CH_3; (2*R,* 3*S*), 6H, m, 2 x CH_3); δ_H (700 MHz, DMSO-d_6) 7.27 ((2*S,* 3*S*), 1H, br. s., CO_2H; (2*R,* 3*S*), 1H, br. s., CO_2H), 4.64 - 4.50 ((2*S,* 3*S*), 2H, m, NH_2CH; (2*R,* 3*S*), 2H, m, NH_2CH), 3.08 ((2*R,* 3*S*), 1H, d, *J* 2.9 Hz, NHC*H*), 3.05 ((2*S,* 3*S*), 1H, d, *J* 3.2 Hz, NHC*H*) 1.98 - 1.86 ((2*R,* 3*S*), 1H, m, $CHCH_3$), 1.85 - 1.73 ((2*S,* 3*S*), 1H, m, $CHCH_3$), 1.54 - 1.32 ((2*S,* 3*S*), 1H, m, $CHCH_2CH_3$; (2*R,* 3*S*), 1H, m, $CHCH_2CH_3$), 1.22 - 1.12 ((2*S,* 3*S*), 1H, m, $CHCH_2CH_3$; (2*R,* 3*S*), 1H, m, $CHCH_2CH_3$), 0.90 - 0.75 ((2*S,* 3*S*), 6H, m, $CHCH_3$, $CHCH_2CH_3$; (2*R,* 3*S*), 6H, m, $CHCH_3$, $CHCH_2CH_3$); δ_C (100 MHz, D_2O) 171.5 ((2*S,* 3*S*), CO_2H; (2*R,* 3*S*), CO_2H), 57.2, 56.8 ((2*S,* 3*S*), *C*HNH; (2*R,* 3*S*), *C*HNH), 35.7, 35.3 ((2*S,* 3*S*), $CHCH_3$; (2*R,* 3*S*), $CHCH_3$), 25.0, 24.7 ((2*S,* 3*S*), CH_2CH_3; (2*R,* 3*S*), CH_2CH_3), 14.1, 13.3 ((2*S,* 3*S*), $CHCH_3$; (2*R,* 3*S*), $CHCH_3$) 10.9, 10.8 ((2*S,* 3*S*), CH_2CH_3; (2*R,* 3*S*), CH_2CH_3); *m/z* (ESI+) 132.1 ([M+H+], 100%).

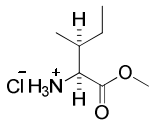
L-Isoleucine methyl ester hydrochloride and D-*allo*-isoleucine methyl ester hydrochloride 3.12



Acetyl chloride (4.5 mL, 63 mmol) was added dropwise to stirred and cooled MeOH (30 ml) to give a solution of methanolic hydrochloric acid. To this was added a mixture of L-isoleucine and D-*allo*-isoleucine **3.11** (76:24 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR; 1.9 g, 16 mmol) and the reaction mixture was heated to reflux for 18 hours. The resulting clear, colourless solution was concentrated *in vacuo* to give the methyl ester hydrochloride salt (2.8 g, 99%, 67:33 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR); υ_{max}/cm^{-1}(neat) 2963 (br. NH), 1742 (C=O); δ_H (400 MHz, CD_3OD) 4.04 ((2*R,* 3*S*), 1H, *J* 4.0 Hz, NH_3CH), 4.02 ((2*S,* 3*S*), 1H, d, *J* 4.0 Hz, NH_3CH), 3.85 ((2*S,* 3*S*), 3H, s, OCH_3; (2*R,* 3*S*), 3H, s, OCH_3), 2.10 - 1.96 ((2*S,* 3*S*), 1H, m, $CHCH_3$; (2*R,* 3*S*), 1H, m, $CHCH_3$), 1.68 - 1.46 ((2*S,* 3*S*), 1H, m, CH_2CH_3; (2*R,* 3*S*), 1H, m, CH_2CH_3), 1.47 - 1.23 ((2*S,* 3*S*), 1H, m, CH_2CH_3; (2*R,* 3*S*), 1H, m, CH_2CH_3), 1.03 ((2*R,* 3*S*), 3H, d, *J* 7.0 Hz, $CHCH_3$), 1.02 ((2*S,* 3*S*), 1H, d, *J* 7.0 Hz, $CHCH_3$), 1.00 ((2*S,* 3*S*), 3H, t, *J* 7.5 Hz, CH_2CH_3; (2*S,* 3*S*), 3H, t, *J* 7.5 Hz, CH_2CH_3); δ_H (700 MHz, DMSO-d_6) 8.55 ((2*S,* 3*S*), 3H, br. s., NH_3CH; (2*R,* 3*S*), 3H, br. s., NH_3CH), 3.92 ((2*R,* 3*S*), 1H, d, *J* 3.8 Hz, NH_3CH), 3.90 ((2*S,* 3*S*), 1H, d, *J* 4.2 Hz, NH_3CH), 3.78 - 3.71 ((2*S,* 3*S*), 3H, m, OCH_3; (2*R,* 3*S*), 3H, m, OCH_3), 1.98 - 1.87 ((2*S,* 3*S*), 1H, m, $CHCH_3$; (2*R,* 3*S*), 1H, m, $CHCH_3$), 1.53 ((2*R,* 3*S*), 1H, dquin, *J* 13.1, 6.7 Hz, $CHCH_2CH_3$), 1.46 ((2*S,* 3*S*), 1H, dquin, *J* 13.6, 6.8 Hz, $CHCH_2CH_3$), 1.27 ((2*S,* 3*S*), 1H, dquin, *J* 15.4, 7.7 Hz, $CHCH_2CH_3$), 1.11 ((2*R,* 3*S*), 1H, dqd, *J* 14.7, 7.7, 5.8 Hz, $CHCH_2CH_3$), 0.95 - 0.85 ((2*S,* 3*S*), 6H, m, $CHCH_2CH_3$, $CHCH_3$; (2*R,* 3*S*), 6H, m, $CHCH_2CH_3$, $CHCH_3$); δ_C (100 MHz, CD_3OD) 170.2 ((2*S,* 3*S*), CO_2CH_3; (2*R,* 3*S*), CO_2CH_3), 58.2, ((2*S,* 3*S*), NH_3CH; (2*R,* 3*S*), NH_3CH), 53.7 ((2*S,* 3*S*), OCH_3; (2*R,* 3*S*), OCH_3), 37.9, 37.5 ((2*S,* 3*S*), $CHCH_3$; (2*R,* 3*S*), $CHCH_3$), 26.8, 26.3 ((2*S,* 3*S*), CH_2CH_3; (2*R,* 3*S*), CH_2CH_3),

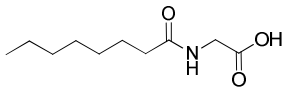
15.0, 14.9 ((2*S,* 3*S*), CH*C*H$_3$; (2*R,* 3*S*), CH*C*H$_3$), 12.1 ((2*S,* 3*S*), CH$_2$*C*H$_3$; (2*R,* 3*S*), CH$_2$*C*H$_3$); *m/z* (ESI+) 146.1 ([M+H], 100%); HR-ESIMS: calculated for $C_7H_{17}NO_2$: 146.1176, found 146.1176 $[M\text{-}Cl]^+$.

L-Isoleucine methyl ester hydrochloride 3.13



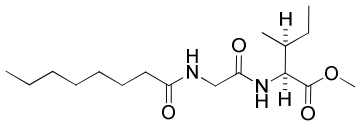
Acetyl chloride (15.6 mL, 220 mmol) was added dropwise to stirred and cooled MeOH (100 ml) to give a solution of methanolic hydrochloric acid. To this was added L-isoleucine (6.54 g, 54.9 mmol) and the reaction mixture was heated to reflux for 18 hours. The resulting clear, colourless solution was concentrated *in vacuo* to give the methyl ester hydrochloride salt as a sticky white gum (9.91 g, 54.6 mmol, 99%); υ_{max}/cm^{-1}(neat) 2940 (br. N-H), 1736 (C=O); $[\alpha]_D^{25}$ +35.8 (c = 0.98, MeOH), (lit.[8] $[\alpha]_D^{20}$ +37.8 (c = 2.0, MeOH)) δ_H (400 MHz, CD_3OD) 4.02 (1H, d, *J* 4.0 Hz, C*H*NH$_3$), 3.84 (3H, s, OC*H*$_3$), 2.16 - 1.89 (1H, m, C*H*CH$_3$), 1.67 - 1.48 (1H, m, C*H*$_2$CH$_3$), 1.46 - 1.26 (1H, m, C*H*$_2$CH$_3$), 1.02 (3H, d, *J* 7.0 Hz, CHC*H*$_3$), 0.99 (3H, t, *J* 7.5 Hz, CH$_2$C*H*$_3$); δ_H (700 MHz, DMSO-d_6) 8.52 (3H, br. s., N*H*$_3$CH), 3.91 (1H, d, *J* 4.2 Hz, NH$_3$C*H*), 3.75 (3H, s, OC*H*$_3$), 1.96 - 1.86 (1H, m, C*H*CH$_3$), 1.46 (1H, dquin, *J* 13.5, 7.1 Hz, CHC*H*$_2$CH$_3$), 1.27 (1H, dquin, *J* 14.7, 7.4 Hz, CHC*H*$_2$CH$_3$), 0.94 - 0.84 (6H, CHC*H*$_3$, CHC*H*$_2$CH$_3$); δ_C (100 MHz, CD_3OD) 170.2 (*C*O$_2$CH$_3$), 58.2 (NH$_3$*C*H), 53.3 (O*C*H$_3$), 37.7 (*C*HCH$_3$), 26.6 (*C*H$_2$CH$_3$), 14.9 (CH*C*H$_3$), 11.9 (CH$_2$*C*H$_3$); *m/z* (ESI+) 146.1 ([M-Cl], 100%); HR-ESIMS: calculated for $C_7H_{17}NO_2$: 146.1176, found 146.1177 $[M\text{-}Cl]^+$. The data are consistent with that previously reported.[8]

Octanoyl glycine 3.14



A solution of octanoyl chloride (17.0 mL, 99.6 mmol) in THF (100 mL) was added dropwise to a stirred solution of glycine (9.0 g, 120 mmol), KOH (6.73 g, 120 mmol) and K_2CO_3 (16.6 g, 120 mmol) in water (250 mL). The reaction mixture was stirred at room temperature for 72 hours. The reaction mixture was acidified with conc. H_2SO_4 (~10.7 mL) and water (100 mL) was added. The reaction mixture was reduced *in vacuo* and filtered. The filtercake was washed with water (4 x 100 mL) and dried under vacuum to give a bright white powdery solid (17.2 g, 85.7 mmol, 86%); m.p. 105-106 °C (lit.[9] 103 - 105 °C); υ_{max}/cm^{-1} (neat) 3310 (N-H), 1708 (acid C=O), 1641 (amide C=O), 1543 (N-H); δ_H (400 MHz, $CDCl_3$) 6.14 (1H, br. s., N*HC*H_2), 4.09 (2H, d, *J* 5.0 Hz, NHC*H*$_2$), 2.28 (2H, t, *J* 7.5 Hz, C*H*$_2$CONH), 1.65 (2H, quin, *J* 7.5 Hz, C*H*$_2$CH$_2$CONH), 1.38 - 1.22 (8H, m, 4 x C*H*$_2$), 0.89 (3H, t, *J* 6.5 Hz, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 173.1, 172.8 (*C*ONH, *C*O_2H), 41.5 (NH*C*H_2), 36.3 (*C*H_2CONH), 31.7, 29.2, 29.0 (3 x *C*H_2), 25.5 (*C*H_2CH$_2$CONH), 22.6 (*C*H_2), 14.1 (*C*H_3); *m/z* (ESI+) 224.0 ([M+Na]; HR-ESIMS: calculated for $C_{10}H_{19}NO_3Na$: 224.1257, found 224.1255 $[M+Na]^+$. The data are consistent with that previously reported.[10]

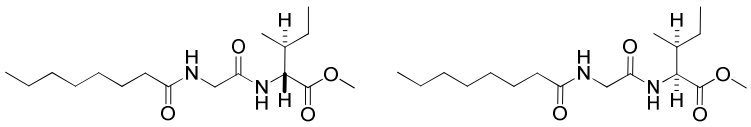
Octanoylglycyl-L-isoleucine methyl ester 3.15



A solution of L-isoleucine methyl ester hydrochloride **3.13** (6.9 g, 38.1 mmol) and N-methylmorpholine (4.1 mL, 38.1 mmol) in EtOH (75 mL) was added dropwise to a solution of octanoyl-glycine (7.45 g, 37.0 mmol) and HOBt (88%, 0.85 g, 5.55 mmol) in EtOH (100 mL). The reaction mixture was cooled to 0 - 5 °C and *N*-methylmorpholine (8.2 mL, 81.4 mmol) was added. The reaction mixture was stirred at 0-5 °C for 15 minutes, EDCI (8.49 g, 44.4 mmol) was added and the reaction allowed to reach room temperature overnight. The reaction was acidified with pH 2 buffer and filtered. The filtrate was reduced *in vacuo* and EtOAc (200 mL) was added. The phases were separated and the organic phase was washed with pH 2 buffer (100 mL), water (100 mL), saturated aqueous $NaHCO_3$ solution (100 mL) and saturated aqueous NaCl solution (100 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The dipeptide was obtained by silica chromatograohy (EtOAc) as a white low melting point solid (5.7 g, 17.3 mmol, 47%); $[\alpha]_D^{25}$ -9.47 (c = 1.0, MeOH); υ_{max}/cm^{-1} (neat) 3296 (N-H), 1744 (ester C=O), 1640 (amide C=O), 1534 (N-H); δ_H (400 MHz, $CDCl_3$) 7.24 (1H, d, *J* 8.5 Hz, N*H*CH), 6.76 (1H, t, *J* 5.0 Hz, N*H*CH_2), 4.52 (1H, dd, *J* 8.0, 5.0 Hz, NHC*H*), 4.00 (2H, d, *J* 5.0 Hz, NHCH_2), 3.71 (3H, s, OCH_3), 2.22 (2H, t, *J* 8.0 Hz, COCH_2H_2), 1.94 - 1.83 (1H, m, Ile C*H*CH_3), 1.61 (2H, quin, *J* 7.5 Hz, CH_2CH_2CONH), 1.41 (1H, dqd, *J* 13.0, 7.5, 5.0 Hz, Ile CH_2CH_3), 1.35 - 1.12 (9H, m, Ile CH_2CH_3, 4 x CH_2), 0.90 (3H, d, *J* 7.0 Hz, Ile CHCH_3), 0.88 - 0.83 (6H, m, Ile CH_2CH_3, CH_2CH_3); δ_H (700 MHz, DMSO-d_6) 8.08 (1H, d, *J* 7.9 Hz, N*H*CH), 7.98 (1H, t, *J* 5.7 Hz, N*H*CH_2), 4.23 (1H, dd, *J* 8.1, 6.4 Hz, NHC*H*), 3.75 (1H, dd, *J* 16.3, 5.7 Hz, NHCH_2), 3.71 (1H, dd, *J* 16.7, 6.2 Hz, NHCH_2), 3.67 - 3.62 (3H, m, OCH_3), 2.10 (2H, t, *J* 7.3 Hz, NHCOCH_2), 1.80 – 1.74 (1H, m, C*H*CH_3), 1.47 (2H, quin, *J* 7.3 Hz, $COCH_2$CH_2), 1.38 (1H, dqd, *J* 14.9, 7.5, 4.2 Hz, CHCH_2CH_3), 1.30 - 1.19 (8H, m, 4 x CH_2), 1.19 - 1.12 (1H, m, CHCH_2CH_3), 0.87 - 0.81 (9H, m,

CHC*H*$_3$, CHCH$_2$C*H*$_3$, CH$_2$CH$_2$C*H*$_3$); δ_H (700 MHz, CD_3OD) 4.41 (1H, d, *J* 5.7 Hz, NHC*H*), 3.92 (1H, d, *J* 16.7 Hz, NHC*H*$_2$), 3.85 (1H, d, *J* 16.7 Hz, NHC*H*$_2$), 3.71 (3H, s, OC*H*$_3$), 2.25 (2H, t, *J* 7.7 Hz, *CH*$_2$*C*H$_2$CO), 1.91 - 1.85 (1H, m, C*H*CH$_3$), 1.62 (2H, quin, *J* 7.4 Hz, COCH$_2$C*H*$_2$), 1.47 (1H, dqd, *J* 15.0, 7.4, 4.6 Hz, CHC*H*$_2$CH$_3$), 1.35 - 1.27 (8H, m, 4 x C*H*$_2$), 1.26 - 1.19 (1H, m, CHC*H*$_2$CH$_3$), 0.95 - 0.87 (9H, m, CHC*H*$_3$, CHCH$_2$C*H*$_3$, CH$_2$CH$_2$C*H*$_3$) δ_C (100 MHz, $CDCl_3$) 174.0 (CH$_2$CH$_2$*C*ONH), 172.1 (*C*O$_2$Me), 169.3 (HNCH$_2$*C*ONH), 56.7 (Ile NH*C*H), 52.1 (O*C*H$_3$), 43.3 (CH$_2$*C*H$_2$CONH), 37.6 (*C*HCH$_3$), 36.3 (NH*C*H$_2$CONH), 31.7, 29.3, 29.0 (3 x *C*H$_2$), 25.7, 25.3, 22.6 (*C*H$_2$CH$_2$CONH, CH*C*H$_2$CH$_3$, *C*H$_2$), 15.5, 14.1 (CH$_2$CH$_2$*C*H$_3$, CH*C*H$_3$), 11.5 (CHCH$_2$*C*H$_3$); *m/z* (ESI+) 351.2 ([M+Na]; HR-ESIMS: calculated for $C_{17}H_{32}N_2O_4Na$: 351.2254, found 351.2252 $[M+Na]^+$.

Octanoylglycyl-L-isoleucine methyl ester and octanoylglycyl-D-*allo*-isoleucine methyl ester 3.17

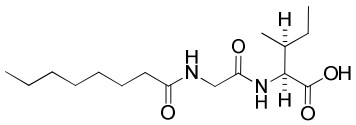


Prepared as for the single diastereomer, using a mix of L- and D-*allo*isoleucine methyl ester **3.12** (2.76 g, 15.2 mmol) and octanoylglycine (2.98 g, 14.8 mmol) to give dipeptide **3.17** as a clear oil (1.61 g, 4.90 mmol, 33 %, 76:24 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR); υ_{max}/cm^{-1} (neat) 3297 (N-H), 1744 (ester C=O), 1641 (amide C=O), 1533 (N-H); δ_H (400 MHz, $CDCl_3$) 7.17 ((2*S,* 3*S*), 1H, d, *J* 8.5 Hz, N*H*CH), 7.13 ((2*R,* 3*S*), 1H, d, *J* 9.5 Hz, N*H*CH), 6.73 - 6.66 ((2*S,* 3*S*), 1H, m, N*H*CH$_2$; (2*R,* 3*S*), 1H, m, N*H*CH$_2$), 4.64 ((2*R,* 3*S*), 1H, dd, *J* 9.0, 4.5 Hz, NHC*H*), 4.53 ((2*S,* 3*S*), 1H, dd, *J* 8.5, 5.0 Hz, NHC*H*), 4.03 - 3.98 ((2*S,* 3*S*), 2H, m, NHC*H*$_2$; (2*R,* 3*S*), 2H, m, NHC*H*$_2$), 3.72 ((2*S,* 3*S*), 3H, s, OC*H*$_3$; (2*R,* 3*S*), 3H, s, OC*H*$_3$), 2.23 ((2*S,* 3*S*), 2H, t, *J* 7.5 Hz, CH$_2$C*H*$_2$CONH; (2*R,* 3*S*), 2H, t, *J* 7.5 Hz, CH$_2$C*H*$_2$CONH), 1.99 - 1.84 ((2*S,* 3*S*), 1H, m, C*H*CH$_3$; (2*R,* 3*S*), 1H, m, C*H*CH$_3$), 1.62 ((2*S,* 3*S*), 2H, quin, *J* 7.5 Hz, C*H*$_2$CH$_2$CONH; (2*R,* 3*S*), 2H, quin, *J*

7.5 Hz, C*H*$_2$CH$_2$CONH), 1.47 - 1.34 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, CHC*H*$_2$CH$_3$), 1.33 - 1.23 ((2*S,* 3*S*), 9H, m, 4 x C*H*$_2$, CHC*H*$_2$CH$_3$; (2*R,* 3*S*), 9H, m, 4 x C*H*$_2$, CHC*H*$_2$CH$_3$), 0.94 - 0.84 ((2*S,* 3*S*), 9H, m, (CH$_2$)$_2$C*H*$_3$, CHC*H*$_3$, CHCH$_2$C*H*$_3$; (2*R,* 3*S*), 9H, m, (CH$_2$)$_2$C*H*$_3$, CHC*H*$_3$, CHCH$_2$C*H*$_3$); δ_H (700 MHz, DMSO-d$_6$) 8.08 ((2*S,* 3*S*), 1H, d, *J* 8.4 Hz, N*H*CH), 8.02 - 7.95 ((2*S,* 3*S*), 1H, m, N*H*CH$_2$; (2*R,* 3*S*), 2H, m, N*H*CH, N*H*CH$_2$), 4.39 ((2*R,* 3*S*), 1H, dd, *J* 8.6, 5.1 Hz, NHC*H*), 4.23 ((2*S,* 3*S*), 1H, dd, *J* 8.4, 6.6 Hz, NHC*H*), 3.79 - 3.68 ((2*S,* 3*S*), 2H, m, NHC*H*$_2$; (2*R,* 3*S*), 2H, m, NHC*H*$_2$), 3.65 - 3.61 ((2*S,* 3*S*), 3H, m, OC*H*$_3$; (2*R,* 3*S*), 3H, m, OC*H*$_3$), 2.13 - 2.06 ((2*S,* 3*S*), 2H, m, CH$_2$C*H*$_2$CO; (2*R,* 3*S*), 2H, m, CH$_2$C*H*$_2$CO), 1.87 - 1.80 ((2*R,* 3*S*), 1H, m, C*H*CH$_3$), 1.80 – 1.74 ((2*S,* 3*S*), 1H, m, C*H*CH$_3$), 1.48 ((2*S,* 3*S*), 2H, quin, *J* 6.9 Hz, C*H*$_2$CH$_2$CONH; (2*R,* 3*S*), 2H, quin, *J* 6.9 Hz, C*H*$_2$CH$_2$CONH), 1.38 ((2*S,* 3*S*), 1H, dqd, *J* 15.0, 7.5, 4.4 Hz, CHC*H*$_2$CH$_3$), 1.30 - 1.19 ((2*S,* 3*S*), 8H, m, 4 x C*H*$_2$; (2*R,* 3*S*), 9H, m, CHC*H*$_2$CH$_3$, 4 x C*H*$_2$), 1.19 - 1.08 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, CHC*H*$_2$CH$_3$), 0.87 - 0.81 ((2*S,* 3*S*), 9H, m, CH$_2$CH$_2$C*H*$_3$, CHC*H*$_3$, CHCH$_2$C*H*$_3$; (2*R,* 3*S*), 9H, m, CH$_2$CH$_2$C*H*$_3$, CHC*H*$_3$, CHCH$_2$C*H*$_3$); δ_H (700 MHz, CD$_3$OD) 4.56 ((2*R*, 3*S*), 1H, d, *J* 4.5 Hz, NHC*H*), 4.42 ((2*S*, 3*S*), 1H, d, *J* 6.2 Hz, NHC*H*), 3.95 - 3.83 ((2*S*, 3*S*), 2H, m, NHC*H*$_2$; (2*R*, 3*S*), 2H, m, NHC*H*$_2$), 3.72 ((2*R*, 3*S*), 3H, s, OC*H*$_3$) 3.71 ((2*S*, 3*S*), 3H, s, OC*H*$_3$), 2.26 ((2*S*, 3*S*), 2H, t, *J* 7.5 Hz, CH$_2$C*H*$_2$CO; (2*R*, 3*S*), 2H, t, *J* 7.5 Hz, CH$_2$C*H*$_2$CO), 2.00 - 1.92 ((2*R*, 3*S*), 1H, m, C*H*CH$_3$), 1.91 - 1.84 ((2*S*, 3*S*), 1H, m, C*H*CH$_3$), 1.62 ((2*S*, 3*S*), 2H, quin, *J* 7.4 Hz, C*H*$_2$CH$_2$CO; (2*R*, 3*S*), 2H, quin, *J* 7.4 Hz, C*H*$_2$CH$_2$CO), 1.47 ((2*S*, 3*S*), 1H, dqd, *J* 13.6, 7.5, 4.4 Hz, CHC*H*$_2$CH$_3$) 1.43 - 1.37 ((2*R*, 3*S*), 1H, m, CHC*H*$_2$CH$_3$), 1.36 - 1.27 ((2*S*, 3*S*), 8H, m, 4 x C*H*$_2$; (2*R*, 3*S*), 9H, m, 4 x C*H*$_2$, CHC*H*$_2$CH$_3$), 1.25 - 1.17 ((2*S*, 3*S*), 1H, m, CHC*H*$_2$CH$_3$) 0.96 - 0.88 ((2*S*, 3*S*), 9H, m, 3 x C*H*$_3$; (2*R*, 3*S*), 9H, m, 3 x C*H*$_3$); δ_C (100 MHz, CDCl$_3$) 174.0 ((2*S,* 3*S*), CH$_2$CH$_2$CONH; (2*R,* 3*S*), CH$_2$CH$_2$CONH), 172.4, 172.1 ((2*S,* 3*S*), *C*O$_2$Me; (2*R,* 3*S*), *C*O$_2$Me), 169.5, 169.3 ((2*S,* 3*S*), NHCH$_2$*C*ONH; (2*R,* 3*S*), NHCH$_2$*C*ONH), 56.7, 55.6 ((2*S,* 3*S*), NH*C*H; (2*R,* 3*S*), NH*C*H), 52.2, 52.1 ((2*S,* 3*S*), O*C*H$_3$; (2*R,* 3*S*), O*C*H$_3$), 43.4, 43.3 ((2*S,* 3*S*), NH*C*H$_2$CONH; (2*R,* 3*S*), NH*C*H$_2$CONH), 37.6, 37.4

((2*S,* 3*S*), *C*HCH$_3$; (2*R,* 3*S*), *C*HCH$_3$), 36.4 ((2*S,* 3*S*), CH$_2$*C*H$_2$CONH; (2*R,* 3*S*), CH$_2$*C*H$_2$CONH), 31.7, 29.3, 29.0 ((2*S,* 3*S*), *C*H$_2$; (2*R,* 3*S*), *C*H$_2$), 25.7, 25.7, 25.0, 22.6 ((2*S,* 3*S*), *C*H$_2$CH$_2$CONH, CH*C*H$_2$CH$_3$, *C*H$_2$; (2*R,* 3*S*), *C*H$_2$CH$_2$CONH, CH*C*H$_2$CH$_3$, *C*H$_2$), 15.5, 14.6, 14.1 ((2*S,* 3*S*), (CH$_2$)$_2$*C*H$_3$, CH*C*H$_3$; (2*R,* 3*S*), (CH$_2$)$_2$*C*H$_3$, CH*C*H$_3$), 11.7, 11.5 ((2*S,* 3*S*), CHCH$_2$*C*H$_3$; (2*R,* 3*S*), CHCH$_2$*C*H$_3$); *m/z* (ESI$^+$) 351.2 ([M+Na]; HR-ESIMS: calculated for $C_{17}H_{32}N_2O_4Na$: 351.2254, found 351.2253 [M+Na]$^+$.

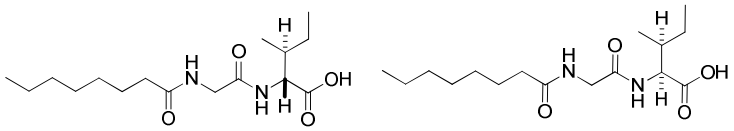
Octanoylglycyl-L-isoleucine 3.16



LiOH (1.66 g, 69.2 mmol) was added to a biphasic solution of the dipeptide **3.15** (5.70 g, 17.3 mmol) in THF (30 mL) and water (30 mL), and the mixture was stirred at room temperature for 4 hours. The reaction mixture was acidified and reduced *in vacuo*. EtOAc (100 mL) was added and the phases were separated. The aqueous phase was further extracted with EtOAc (4 x 10 mL), and the combined organics were washed with water (50 mL) and saturated aqueous NaCl solution (50 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the acid as a bright white solid (4.51 g, 14.3 mmol, 83 %); m.p. 98 - 99 °C; $[\alpha]_D^{25}$ +7.01 (c = 2.0, MeOH)) υ_{max}/cm^{-1} (neat) 3297 (N-H), 1717 (acid C=O), 1640 (amide C=O), 1519 (N-H); δ_H (400 MHz, $CDCl_3$) 9.72 (1H, br. s., CO_2H), 7.37 (1H, d, *J* 8.5 Hz, N*H*CH), 7.13 (1H, t, *J* 5.0 Hz, N*H*CH$_2$), 4.53 (1H, dd, *J* 8.5, 5.0 Hz, NHC*H*), 4.10 (1H, dd, *J* 16.5, 5.5 Hz, NHC*H*$_2$), 3.98 (1H, dd, *J* 16.5, 5.0 Hz, NHC*H*$_2$), 2.25 (2H, t, *J* 8.0 Hz, CH$_2$C*H*$_2$CO), 2.00 - 1.87 (1H, m, C*H*CH$_3$), 1.67 - 1.57 (2H, m, *C*H$_2$CH$_2$CO), 1.56 - 1.42 (1H, m, CHC*H*$_2$CH$_3$), 1.34 - 1.14 (9H, m, CHC*H*$_2$CH$_3$, 4 x C*H*$_2$), 0.96 - 0.84 (9H, m, CHCH$_2$C*H*$_3$, CHC*H*$_3$, CH$_2$CH$_2$C*H*$_3$); δ_H (700 MHz, DMSO-d_6) 12.63 (1H, br. s., CO_2H), 8.00 (1H, t, *J* 5.9 Hz, N*H*CH$_2$), 7.87 (1H, d, *J* 8.8 Hz, NHC*H*), 4.19 (1H, dd, *J* 8.6, 5.9 Hz, NHC*H*), 3.75 (1H, dd, *J* 16.7, 5.7 Hz, NHC*H*$_2$), 3.71 (1H, dd, *J* 15.8, 5.7 Hz, NHC*H*$_2$), 2.10 (2H, t, *J* 7.5 Hz, COC*H*$_2$CH$_2$), 1.82 - 1.73 (2H, m, C*H*CH$_3$),

1.48 (2H, quin, *J* 7.2 Hz, $COCH_2CH_2$), 1.39 (1H, dqd, *J* 15.0, 7.5, 4.8 Hz, $CHCH_2CH_3$), 1.30 - 1.19 (8H, m, 4 x CH_2), 1.19 - 1.11 (1H, m, $CHCH_2CH_3$), 0.87 - 0.82 (9H, m, $CH_2CH_2CH_3$, $CHCH_3$, $CHCH_2CH_3$); δ_H (700 MHz, CD_3OD) 4.40 (1H, d, *J* 5.3 Hz, NHCH), 3.93 (1H, d, *J* 16.3 Hz, $NHCH_2$), 3.86 (1H, d, *J* 16.7 Hz, $NHCH_2$), 2.26 (2H, t, *J* 7.5 Hz, $COCH_2CH_2$), 1.95 - 1.86 (1H, m, $CHCH_3$), 1.67 - 1.58 (2H, m, $COCH_2CH_2$), 1.55 - 1.48 (1H, m, $CHCH_2CH_3$), 1.37 - 1.27 (8H, m, 4 x CH_2), 1.26 - 1.18 (1H, m, $CHCH_2CH_3$), 0.96 - 0.85 (9H, m, $CHCH_2CH_3$, $CHCH_3$, $CH_2CH_2CH_3$); δ_C (100 MHz, $CDCl_3$) 174.9, 174.4 (2 x *C*ONH), 169.9 (CO_2H), 56.9 (*C*HNH), 43.1 ($NHCH_2$), 37.4 ($CHCH_3$), 36.3 (CH_2CH_2CO), 31.7, 29.2, 29.0, 25.7, 25.0, 22.6 ($CHCH_2CH_3$, 5 x CH_2), 15.4, 14.1, 11.6 (2 x CH_2CH_3, $CHCH_3$); *m/z* (ESI+) 337.1 ([M+Na]; HR-ESIMS: calculated for $C_{16}H_{30}N_2O_4Na$: 337.2098, found 337.2097 $[M+Na]^+$.

Octanoylglycyl-L-isoleucine and octanoylglycyl-D-*allo*-isoleucine 3.18

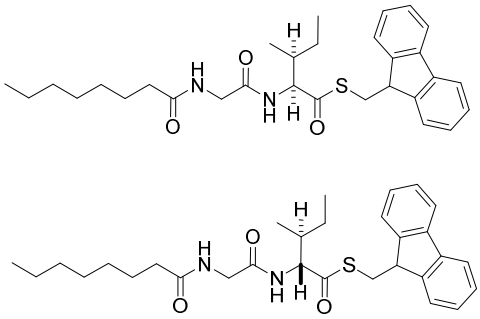


Prepared as for the single diastereomer, using a mix of L- and D-*allo*-isoleucine dipeptide **3.17** (1.61 g, 4.89 mmol) to give the acid as a bright white solid (1.34 g, 4.26 mmol, 87 %, 70:30 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR); υ_{max}/cm^{-1} (neat) 3285 (N-H), 1718 (acid C=O), 1636 (amide C=O), 1557 (N-H); δ_H (400 MHz, DMSO-d_6) 12.63 ((2*S,* 3*S*), 1H, br. s., CO_2H; (2*R,* 3*S*), 1H, br. s., CO_2H), 8.06 - 7.96 ((2*S,* 3*S*), 1H, m, $NHCH_2$; (2*R,* 3*S*), 1H, m, $NHCH_2$), 7.86 ((2*S,* 3*S*), 1H, d, *J* 8.5 Hz, NHCH), 7.79 ((2*R,* 3*S*), 1H, d, *J* 9.0 Hz, NHCH), 4.35 ((2*R,* 3*S*), 1H, dd, *J* 8.5, 4.5 Hz, NHCH), 4.19 ((2*S,* 3*S*), 1H, dd, *J* 8.5, 6.0 Hz, NHCH), 3.80 - 3.67 ((2*S,* 3*S*), 2H, m, $NHCH_2$; (2*R,* 3*S*), 2H, m, $NHCH_2$), 2.10 ((2*S,* 3*S*), 2H, t, *J* 7.5 Hz, CH_2CH_2CO; (2*R,* 3*S*), 2H, t, *J* 7.5 Hz, CH_2CH_2CO), 1.90 - 1.70 ((2*S,* 3*S*), 1H, m, $CHCH_3$; (2*R,* 3*S*), 1H, m, $CHCH_3$), 1.48 ((2*S,* 3*S*), 2H, quin, *J* 7.5 Hz, CH_2CH_2CO; (2*R,* 3*S*), 2H, quin, *J* 7.5 Hz, CH_2CH_2CO), 1.43 - 1.33 ((2*S,* 3*S*), 1H, m, $CHCH_2CH_3$; (2*R,* 3*S*), 1H, m, $CHCH_2CH_3$), 1.31 - 1.20 ((2*S,* 3*S*), 8H, m, 4 x

C*H*$_2$; (2*R,* 3*S*), 8H, m, 4 x C*H*$_2$), 1.19 - 1.04 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, CHC*H*$_2$CH$_3$), 0.89 - 0.80 ((2*S,* 3*S*), 9H, m, CH$_2$CH$_2$C*H*$_3$, CHC*H*$_3$, CHCH$_2$C*H*$_3$; (2*R,* 3*S*), 9H, m, CH$_2$CH$_2$C*H*$_3$, CHC*H*$_3$, CHCH$_2$C*H*$_3$); δ_H (700 MHz, DMSO-d$_6$) 12.65 ((2*S,* 3*S*), 1H, br. s., CO$_2$*H*; (2*R,* 3*S*), 1H, br. s., CO$_2$*H*), 8.03 - 7.99 ((2*S,* 3*S*), 1H, m, N*H*CH$_2$; (2*R,* 3*S*), 1H, m, N*H*CH$_2$), 7.87 ((2*S,* 3*S*), 1H, d, *J* 8.8 Hz, N*H*CH), 7.79 ((2*R,* 3*S*), 1H, d, *J* 8.8 Hz, N*H*CH), 4.36 ((2*R,* 3*S*), 1H, dd, *J* 8.8, 4.4 Hz, NHC*H*), 4.19 ((2*S,* 3*S*), 1H, dd, *J* 8.6, 5.9 Hz, NHC*H*), 3.78- 3.68 ((2*S,* 3*S*), 2H, m, NHC*H*$_2$; (2*R,* 3*S*), 2H, m, NHC*H*$_2$), 2.10 ((2*S,* 3*S*), 2H, t, *J* 7.5 Hz, COC*H*$_2$CH$_2$; (2*R,* 3*S*), 2H, t, *J* 7.5 Hz, COC*H*$_2$CH$_2$), 1.88 - 1.82 ((2*R,* 3*S*), 1H, m, C*H*CH$_3$), 1.80 - 1.72 ((2*S,* 3*S*), 1H, m, C*H*CH$_3$), 1.48 ((2*S,* 3*S*), 2H, quin, *J* 7.0 Hz, COCH$_2$C*H*$_2$; (2*R,* 3*S*), 2H, quin, *J* 7.0 Hz, COCH$_2$C*H*$_2$), 1.39 ((2*S,* 3*S*), 1H, dqd, *J* 15.0, 7.5, 4.6 Hz, CHC*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, dqd, *J* 15.0, 7.5, 4.6 Hz, CHC*H*$_2$CH$_3$), 1.31 - 1.19 ((2*S,* 3*S*), 8H, 4 x C*H*$_2$; (2*R,* 3*S*), 8H, 4 x C*H*$_2$), 1.19 - 1.10 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, CHC*H*$_2$CH$_3$), 0.90 - 0.81 ((2*S,* 3*S*), 9H, CH$_2$CH$_2$C*H*$_3$, CHC*H*$_3$, CHCH$_2$C*H*$_3$; (2*R,* 3*S*), 9H, CH$_2$CH$_2$C*H*$_3$, CHC*H*$_3$, CHCH$_2$C*H*$_3$); δ_H (700 MHz, CD$_3$OD) 4.55 ((2*R,* 3*S*), 1H, d, *J* 4.0 Hz, NHC*H*), 4.40 ((2*S,* 3*S*), 1H, d, *J* 5.3 Hz, NHC*H*), 3.96 - 3.90 ((2*S,* 3*S*), 1H, m, NHC*H*$_2$; (2*R,* 3*S*), 1H, m, NHC*H*$_2$), 3.89 - 3.84 ((2*S,* 3*S*), 1H, m, NHC*H*$_2$; (2*R,* 3*S*), 1H, m, NHC*H*$_2$), 2.26 ((2*S,* 3*S*), 2H, t, *J* 7.5 Hz, COC*H*$_2$CH$_2$; (2*R,* 3*S*), 2H, t, *J* 7.5 Hz, COC*H*$_2$CH$_2$), 2.01 - 1.95 ((2*R,* 3*S*), 1H, m, C*H*CH$_3$), 1.93 - 1.87 ((2*S,* 3*S*), 1H, m, C*H*CH$_3$), 1.63 ((2*S,* 3*S*), 2H, quin, *J* 7.4 Hz, COCH$_2$C*H*$_2$; (2*R,* 3*S*), 2H, quin, *J* 7.4 Hz, COCH$_2$C*H*$_2$), 1.51 ((2*S,* 3*S*), 1H, dqd, *J* 15.0, 7.5 Hz, 4.4 Hz, CHC*H*$_2$CH$_3$), 1.42 ((2*R,* 3*S*), 1H, dquin, *J* 14.1, 7.2 Hz, CHC*H*$_2$CH$_3$), 1.37 - 1.27 ((2*S,* 3*S*), 8H, m, 4 x C*H*$_2$; (2*R,* 3*S*), 8H, m, 4 x C*H*$_2$), 1.26 - 1.19 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, CHC*H*$_2$CH$_3$), 0.97 - 0.88 ((2*S,* 3*S*), 9H, m, CH$_2$CH$_2$C*H*$_3$, CHC*H*$_3$, CHCH$_2$C*H*$_3$; (2*R,* 3*S*), 9H, m, CH$_2$CH$_2$C*H*$_3$, CHC*H*$_3$, CHCH$_2$C*H*$_3$); δ_H (700 MHz, CDCl$_3$) 7.22 ((2*S,* 3*S*), 1H, d, *J* 7.5 Hz, N*H*CH; (2*R,* 3*S*), 1H, d, *J* 7.5 Hz, N*H*CH), 6.84 ((2*S,* 3*S*), 1H, br. s., N*H*CH$_2$; (2*R,* 3*S*), 1H, br. s., N*H*CH$_2$), 4.67 ((2*R,* 3*S*), 1H, dd, *J* 8.5, 3.5 Hz, NHC*H*), 4.53 ((2*S,* 3*S*), 1H, dd, *J* 7.0, 5.3 Hz, NHC*H*), 4.19 ((2*S,* 3*S*), 1H, dd, *J* 16.7, 5.3 Hz, NHC*H*$_2$;

(2*R,* 3*S*), 1H, dd, *J* 16.7, 5.3 Hz, NHCH_2), 3.90 ((2*S,* 3*S*), 1H, dd, *J* 16.5, 4.6 Hz, NHCH_2; (2*R,* 3*S*), 1H, dd, *J* 16.5, 4.6 Hz, NHCH_2), 2.25 ((2*S,* 3*S*), 2H, t, *J* 7.5 Hz, CH_2CH_2CO; (2*R,* 3*S*), 2H, t, *J* 7.5 Hz, CH_2CH_2CO), 2.05 - 1.87 ((2*S,* 3*S*), 1H, m, C*H*CH_3; (2*R,* 3*S*), 1H, m, C*H*CH_3), 1.63 ((2*S,* 3*S*), 2H, quin., *J* 6.7 Hz, CH_2CH_2CO; (2*R,* 3*S*), 2H, quin., *J* 6.7 Hz, CH_2CH_2CO), 1.53 - 1.46 ((2*S,* 3*S*), 1H, m, $CHCH_2CH_3$; (2*R,* 3*S*), 1H, m, $CHCH_2CH_3$), 1.35 - 1.17 ((2*S,* 3*S*), 9H, m, $CHCH_2CH_3$, 4 x CH_2; (2*R,* 3*S*), 9H, m, $CHCH_2CH_3$, 4 x CH_2) 0.97 - 0.86 ((2*S,* 3*S*), 9H, m, 3 x CH_3; (2*R,* 3*S*), 9H, m, 3 x CH_3); δ_C (100 MHz, DMSO) 174.3 ((2*S,* 3*S*), CH_2CH_2CONH; (2*R,* 3*S*), CH_2CH_2CONH), 172.8, 172.5 ((2*S,* 3*S*), CO_2H; (2*R,* 3*S*), CO_2H), 169.1 ((2*S,* 3*S*), $NHCH_2CONH$; (2*R,* 3*S*), $NHCH_2CONH$), 56.1 ((2*S,* 3*S*), NHCH), 54.6 ((2*R,* 3*S*), NHCH), 41.7 ((2*S,* 3*S*), CH_2CH_2CONH; (2*R,* 3*S*), CH_2CH_2CONH), 36.5 ((2*S,* 3*S*), $CHCH_3$; (2*R,* 3*S*), $CHCH_3$), 35.1 ((2*S,* 3*S*), $NHCH_2CONH$; (2*R,* 3*S*), $NHCH_2$ CONH), 31.1, 28.6, 28.5 ((2*S,* 3*S*), CH_2; (2*R,* 3*S*), CH_2), 25.7, 25.2, 24.6, 22.0 ((2*S,* 3*S*), CH_2CH_2CONH, $CHCH_2CH_3$, CH_2; (2*R,* 3*S*), CH_2CH_2CONH, $CHCH_2CH_3$, CH_2), 15.5, 14.7, 13.9 ((2*S,* 3*S*), $(CH_2)_2CH_3$, $CHCH_3$; (2*R,* 3*S*), $(CH_2)_2CH_3$, $CHCH_3$), 11.5, 11.3 ((2*S,* 3*S*), $CHCH_2CH_3$; (2*R,* 3*S*), $CHCH_2CH_3$); *m/z* (ESI+) 337.1 ([M+Na]; HR-ESIMS: calculated for $C_{16}H_{30}N_2O_4Na$: 337.2098, found 337.2093 $[M+Na]^+$.

***S*-((9H-Fluoren-9-yl)methyl)-(2*S*, 3*S*)-3-methyl-2-(2-octanamidoacetamido)pentanethioate and *S*-((9H-fluoren-9-yl)methyl)-(2*R*, 3*S*)-3-methyl-2-(2-octanamidoacetamido)pentanethioate 3.19**



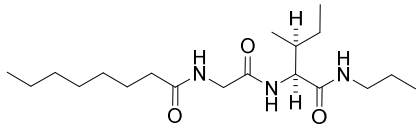
Method modified from literature procedure by Crich *et al.*[1] 4 Å Molecular sieves were added to a solution of 9-fluorenylmethylthiol (0.20 g, 0.94 mmol) and acid **3.16** (0.23 g, 0.72 mmol) in DMF (12 mL), and the mixture was stirred at - 20 °C for 20 minutes. PyBOP (0.94 g, 1.82 mmol) and diisopropylethylamine (0.30 mL, 1.82 mmol) were added and the reaction mixture stirred at -20 °C for 4 hours. The reaction mixture was filtered, quenched with pH 2 buffer (15 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The combined organics were concentrated *in vacuo*. EtOAc (15 mL) and pH 2 buffer (10 mL) were added and the phases were separated. The organics were washed with pH 2 buffer (10 mL), water (3 x 10 mL), saturated aqueous $NaHCO_3$ solution (10mL) and saturated aqueous NaCl solution (10 mL), dried over Na_2SO_4 and concentrated *in vacuo* (88: 12 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers in crude product, calculated by ^{1}H NMR). The thioester **3.19** was obtained by silica column chromatography (0 - 2% MeOH: CH_2Cl_2) as a white solid (87 mg, 0.17 mmol, 28 %, 80: 20 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR); υ_{max}/cm^{-1} (neat) 3261 (N-H), 1689 (C=O), 1672, 1632 (amide C=O), 1521 (N-H); δ_H (400 MHz, $CDCl_3$) 7.73 ((2*S,* 3*S*), 2H, d, *J* 7.5 Hz, 2 x Ar C*H*; (2*R,* 3*S*), 2H, d, *J* 7.5 Hz, 2 x Ar C*H*), 7.60 ((2*S,* 3*S*), 2H, t, *J* 7.0 Hz, 2 x Ar C*H*; (2*R,* 3*S*), 2H, t, *J* 7.0 Hz, 2 x Ar C*H*), 7.42 - 7.35 ((2*S,* 3*S*), 2H, m, 2 x Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x Ar C*H*), 7.33 – 7.27 ((2*S,* 3*S*), 2H, m, 2 x Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x Ar C*H*), 6.95 - 6.87 ((2*S,* 3*S*), 1H, m, N*H*CH; (2*R,* 3*S*), 1H, m, N*H*CH), 6.44 -

6.36 ((2*S,* 3*S*), 1H, m, N*H*CH_2; (2*R,* 3*S*), 1H, m, N*H*CH_2), 4.65 ((2*R,* 3*S*), 1H, dd, *J* 9.0, 4.0 Hz), 4.53 ((2*S,* 3*S*), 1H, dd, *J* 8.5, 5.0 Hz, NHC*H*), 4.17 ((2*S,* 3*S*), 1H, t, *J* 5.5 Hz, C*H*CH_2S; (2*R,* 3*S*), 1H, t, *J* 5.5 Hz, C*H*CH_2S), 4.06 - 3.89 ((2*S,* 3*S*), 2H, m, $NHCH_2$; (2*R,* 3*S*), 2H, m, $NHCH_2$), 3.63 - 3.52 ((2*S,* 3*S*), 2H, m, CHC*H*$_2$S; (2*R,* 3*S*), 2H, m, CHC*H*$_2$S), 2.18 ((2*S,* 3*S*), 2H, t, *J* 8.0 Hz, CH_2CH_2CONH; (2*R,* 3*S*), 2H, t, *J* 8.0 Hz, CH_2CH_2CONH), 2.00 - 1.84 ((2*S,* 3*S*), 1H, m, C*H*CH_3; (2*R,* 3*S*), 1H, m, C*H*CH_3), 1.61 ((2*S,* 3*S*), 2H, quin, *J* 7.0 Hz, CH_2CH_2CONH; (2*R,* 3*S*), 2H, quin, *J* 7.0 Hz, CH_2CH_2CONH), 1.35 - 1.23 ((2*S,* 3*S*), 8H, m, 4 x C*H*$_2$; (2*R,* 3*S*), 8H, m, 4 x C*H*$_2$), 1.19 - 1.07 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$$CH_3$; (2*R,* 3*S*), 1H, m, CHC*H*$_2$$CH_3$), 1.03 - 0.91 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$$CH_3$; (2*R,* 3*S*), 1H, m, CHC*H*$_2$$CH_3$), 0.91 - 0.57 ((2*S,* 3*S*), 9H, m, CHC*H*$_3$, $CHCH_2$C*H*$_3$, CH_2CH_2C*H*$_3$; (2*S,* 3*S*), 9H, m, CHC*H*$_3$, $CHCH_2$C*H*$_3$, CH_2CH_2C*H*$_3$); δ_H (700 MHz, DMSO-d_6) 8.11 ((2*S,* 3*S*), 1H, d, *J* 8.4 Hz, N*H*CH), 8.05 ((2*R,* 3*S*), 1H, d, *J* 8.4 Hz, N*H*CH), 7.97 - 7.92 ((2*S,* 3*S*), 2H, m, N*H*CH_2; (2*R,* 3*S*), 2H, m, N*H*CH_2), 7.86 -7.83 ((2*S,* 3*S*), 2H, m, 2 x Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x Ar C*H*), 7.65 - 7.57 ((2*S,* 3*S*), 2H, m, 2 x Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x Ar C*H*), 7.40 - 7.36 ((2*S,* 3*S*), 2H, m, 2 x Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x Ar C*H*), 7.33 - 7.27 ((2*S,* 3*S*), 2H, m, 2 x Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x Ar C*H*), 4.31 ((2*R,* 3*S*), 1H, dd, *J* 8.6, 5.1 Hz, NHC*H*), 4.29 - 4.26 ((2*S,* 3*S*), 2H, m, C*H*CH_2S; (2*R,* 3*S*), 2H, m, C*H*CH_2S), 4.15 ((2*S,* 3*S*), 1H, dd, *J* 8.4, 6.2 Hz, NHC*H*), 3.76 - 3.57 ((2*S,* 3*S*), 4H, m, CHC*H*$_2$S, NHC*H*$_2$; (2*R,* 3*S*), 4H, m, CHC*H*$_2$S, NHC*H*$_2$), 2.06 ((2*S,* 3*S*), 1H, t, *J* 7.5 Hz, COC*H*$_2$$CH_2$; (2*S,* 3*S*), 1H, t, *J* 7.5 Hz, COC*H*$_2$$CH_2$), 1.71 - 1.64 ((2*R,* 3*S*), 1H, m, C*H*CH_3), 1.63 - 1.55 ((2*S,* 3*S*), 1H, m, C*H*CH_3), 1.45 ((2*S,* 3*S*), 1H, dt, *J* 7.0 Hz, $COCH_2$C*H*$_2$; (2*R,* 3*S*), 1H, quin., *J* 7.0 Hz, $COCH_2$C*H*$_2$), 1.28 - 1.18 ((2*S,* 3*S*), 8H, m, 4 x C*H*$_2$; (2*R,* 3*S*), 8H, m, 4 x C*H*$_2$), 1.09 - 0.97 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$$CH_3$; (2*R,* 3*S*), 1H, m, CHC*H*$_2$$CH_3$), 0.96 - 0.87 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$$CH_3$; (2*R,* 3*S*), 1H, m, CHC*H*$_2$$CH_3$), 0.84 ((2*S,* 3*S*), 3H, t, *J* 7.0 Hz, CH_2CH_2C*H*$_3$; (2*R,* 3*S*), 3H, t, *J* 7.0 Hz, CH_2CH_2C*H*$_3$), 0.70 ((2*R,* 3*S*), 3H, t, *J* 7.5 Hz, $CHCH_2$C*H*$_3$), 0.67 ((2*S,* 3*S*), 3H, t, *J* 7.3 Hz, $CHCH_2$C*H*$_3$), 0.57 ((2*S,* 3*S*), 3H, d, *J* 6.6 Hz, CHC*H*$_3$), 0.52 ((2*R,* 3*S*), 3H, d, *J* 6.6 Hz, CHC*H*$_3$); δ_H (700 MHz, CD_3OD)

7.75 ((2*S,* 3*S*), 2H, d, 7.5 Hz, 2 x Ar C*H*; (2*R,* 3*S*), 2H, d, 7.5 Hz, 2 x Ar C*H*), 7.60 ((2*S,* 3*S*), 2H, t, 7.5 Hz, 2 x Ar C*H*; (2*R,* 3*S*), 2H, t, 7.5 Hz, 2 x Ar C*H*), 7.36 ((2*S,* 3*S*), 2H, t, 7.3 Hz, 2 x Ar C*H*; (2*R,* 3*S*), 2H, t, 7.3 Hz, 2 x Ar C*H*), 7.31 - 7.23 ((2*S,* 3*S*), 2H, m, 2 x Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x Ar C*H*), 4.48 ((2*R,* 3*S*), 1H, d, *J* 4.8 Hz, NHC*H*), 4.33 ((2*S,* 3*S*), 1H, d, *J* 5.7 Hz, NHC*H*), 4.22 ((2*S,* 3*S*), 1H, t, *J* 4.8 Hz, SC*H*CH_2; (2*R,* 3*S*), 1H, t, *J* 4.8 Hz, SC*H*CH_2), 3.86 ((2*S,* 3*S*), 1H, dd, *J* 16.3, 10.6 Hz, NHC*H*$_2$; (2*R,* 3*S*), 1H, dd, *J* 16.3, 10.6 Hz, NHC*H*$_2$), 3.81 - 3.76 ((2*S,* 3*S*), 1H, m, NHC*H*$_2$; (2*R,* 3*S*), 1H, m, NHC*H*$_2$), 3.75 - 3.70 ((2*S,* 3*S*), 1H, m, SCHC*H*$_2$; (2*R,* 3*S*), 1H, m, SCHC*H*$_2$), 3.67 - 3.59 ((2*S,* 3*S*), 1H, m, SCHC*H*$_2$; (2*R,* 3*S*), 1H, m, SCHC*H*$_2$), 2.20 ((2*S,* 3*S*), 2H, t, *J* 7.5 Hz, COC*H*$_2$$CH_2$; (2*R,* 3*S*), 2H, t, *J* 7.5 Hz, COC*H*$_2$$CH_2$), 1.85 - 1.78 ((2*R,* 3*S*), 1H, m, C*H*CH_3), 1.77 - 1.69 ((2*S,* 3*S*), 1H, m, C*H*CH_3), 1.65 - 1.55 ((2*S,* 3*S*), 2H, m, COCH$_2$C*H*$_2$; (2*R,* 3*S*), 2H, m, COC*H*$_2$$CH_2$), 1.38 - 1.24 ((2*S,* 3*S*), 8H, m, 4 x C*H*$_2$; (2*R,* 3*S*), 8H, m, 4 x C*H*$_2$), 1.20 - 1.12 ((2*R,* 3*S*), 1H, m, CHC*H*$_2$$CH_3$), 1.13 - 1.00 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$$CH_3$; (2*R,* 3*S*), 1H, m, CHC*H*$_2$$CH_3$), 0.98 - 0.92 ((2*S,* 3*S*), 1H, m, CHC*H*$_2$$CH_3$), 0.89 ((2*S,* 3*S*), 3H, t, *J* 7.0 Hz, CH_2CH_2C*H*$_3$; (2*R,* 3*S*), 3H, t, *J* 7.0 Hz, CH_2CH_2C*H*$_3$), 0.81 ((2*R,* 3*S*), 3H, t, *J* 7.5 Hz, CHCH$_2$C*H*$_3$), 0.76 ((2*S,* 3*S*), 3H, t, *J* 7.5 Hz, CHCH$_2$C*H*$_3$), 0.70 ((2*S,* 3*S*), 3H, d, *J* 7.0 Hz, CHC*H*$_3$), 0.60 ((2*R,* 3*S*), 3H, d, *J* 7.0 Hz, CHC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 199.9, 198.3 ((2*S,* 3*S*), S*C*O; (2*R,* 3*S*), S*C*O), 174.2, 174.1 ((2*S,* 3*S*), CHNH*C*O; (2*R,* 3*S*), CHNH*C*O), 169.7, 169.5 ((2*S,* 3*S*), CH_2NH*C*O; (2*R,* 3*S*), CH_2NH*C*O), 145.2, 141.2, 141.2 ((2*S,* 3*S*), Ar *C*; (2*R,* 3*S*), Ar *C*), 127.7, 127.1, 124.7, 119.9 ((2*S,* 3*S*), Ar *C*H; (2*R,* 3*S*), Ar *C*H), 63.8, ((2*S,* 3*S*), NH*C*H), 62.3 ((2*R,* 3*S*), NH*C*H), 46.7 ((2*S,* 3*S*), *C*HCH$_2$S; (2*R,* 3*S*), *C*HCH$_2$S), 43.7, 43.6 ((2*S,* 3*S*), NH*C*H$_2$; (2*R,* 3*S*), NH*C*H$_2$), 37.5 ((2*S,* 3*S*), CH*C*HCH$_3$; (2*R,* 3*S*), CH*C*HCH$_3$), 36.3 ((2*S,* 3*S*), CH_2*C*H$_2$CO; (2*R,* 3*S*), CH_2*C*H$_2$CO), 31.9, 31.7, 31.6, 29.3, 29.1, 26.4, 26.2, 26.1, 25.7, 24.1, 22.6 ((2*S,* 3*S*), *C*H$_2$CH$_2$CO, CH*C*H$_2$S, *C*H$_2$; (2*R,* 3*S*), *C*H$_2$CH$_2$CO, CH*C*H$_2$S, *C*H$_2$), 15.5, 14.1, 13.9 ((2*S,* 3*S*), CH_2CH_2*C*H$_3$, CH*C*H$_3$; (2*R,* 3*S*), CH_2CH_2*C*H$_3$, CH*C*H$_3$), 11.7 ((2*S,* 3*S*), CHCH$_2$*C*H$_3$; (2*R,* 3*S*), CHCH$_2$*C*H$_3$); *m/z* (ESI+) 531.2 ([M+Na]; HR-ESIMS: calculated for $C_{30}H_{40}N_2O_3SNa$: 531.2652, found 531.2628 $[M+Na]^+$.

This compound was also prepared using the same method using a mix of L-isoleucine and D-*allo*-isoleucine dipeptide **3.18** (0.20 g, 0.64 mmol) to give the coupled product **3.20** as a bright white solid (38 mg, 0.075 mol, 12 %. 65: 35 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers in crude product, calculated by ^{1}H NMR; 61:39 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers following purification, calculated by ^{1}H NMR); All characterisation data was identical to that obtained above.

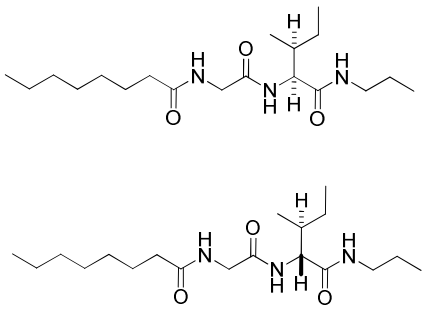
***N*-(Propyl)-(2*S*, 3*S*)-3-methyl-2-(2-octanamidoacetamido)pentaneamide 3.22**



Method modified from literature procedure by Crich *et al.*[1] 4 Å Molecular sieves were added to a solution of propylamine (0.09 mL, 1.34 mmol) and acid **3.17** (0.25 g, 0.80 mmol) in DMF (14 mL), and the mixture was stirred at - 20 °C for 20 minutes. PyBOP (1.0 g, 2.0 mmol) and diisopropylethylamine (0.33 mL, 2.0 mmol) were added and the reaction mixture stirred at - 20 °C for 4 hours. The reaction mixture was filtered, quenched with pH 2 buffer (15 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The combined organics were concentrated *in vacuo*. EtOAc (15 mL) and pH 2 buffer (10 mL) were added and the phases were separated. The organics were washed with pH 2 buffer (10 mL), water (3 x 10 mL), saturated aqueous $NaHCO_3$ solution (10mL) and saturated aqueous NaCl solution (10 mL), dried over Na_2SO_4 and concentrated *in vacuo* (>99 % (2*S*, 3*S*) diastereomer in crude product, calculated by 400 MHz ^{1}H NMR). The product was obtained by silica column chromatography (10% MeOH: CH_2Cl_2) to give **3.22** as a bright white solid (0.21g, 0.59 mmol, 74 %; >99 % (2*S*, 3*S*) diastereomer, calculated by 400 MHz ^{1}H NMR; >93 % (2*S*, 3*S*) diastereomer, calculated by 700 MHz ^{1}H NMR); m.p. 151 - 153 °C; υ_{max}/cm^{-1} (neat) 3294 (N-H), 1629 (C=O), 1532 (N-H); $[\alpha]_D^{25}$ -13.2 (c = 0.42, MeOH)), δ_H (400 MHz, DMSO-d_6) 8.06 (1H, t, *J* 6.0 Hz, N*H*CH_2CO), 7.98 (1H, t, *J* 5.5 Hz, N*H*$CH_2CH_2CH_3$), 7.72 (1H, d, *J* 8.5 Hz, N*H*CH), 4.12 (1H, dd, *J* 9.0 Hz, 7.5 Hz, NHC*H*), 3.73 (1H, dd, *J* 16.5, 5.5 Hz, NHCH_2), 3.67 (1H, dd, *J* 16.5, 5.5 Hz,

NHC*H*$_2$), 3.12 - 2.89 (2H, m, NHC*H*$_2$CH$_2$CH$_3$), 2.10 (2H, t, *J* 7.5 Hz, CH$_2$C*H*$_2$CO), 1.71 - 1.61 (1H, m, C*H*CH$_3$), 1.53 - 1.44 (2H, m, C*H*$_2$CH$_2$CO), 1.44 - 1.32 (3H, m, CHC*H*$_2$, NHCH$_2$C*H*$_2$CH$_3$), 1.29 - 1.21 (8H, m, 4 x C*H*$_2$), 1.11 - 0.98 (1H, m, CHC*H*$_2$), 0.89 - 0.98 (12H, m, CHC*H*$_3$, 3 x CH$_2$C*H*$_3$); δ_H (700 MHz, $CDCl_3$) 7.27 (1H, br. s., N*H*), 6.94 (1H, br. s., N*H*), 6.73 (1H, br. s., N*H*), 4.34 (1H, dd, *J* 8.8, 7.5 Hz, NHC*H*), 4.06 (1H, dd, *J* 17.2, 5.3 Hz, NHC*H*$_2$), 3.97 (1H, dd, *J* 16.3, 4.8 Hz, NHC*H*$_2$), 3.33 - 3.19 (1H, m, NHC*H*$_2$CH$_2$), 3.17 - 3.12 (1H, m, NHC*H*$_2$CH$_2$CH$_3$), 2.29 - 2.24 (2H, t, *J* 8.4 Hz, CH$_2$C*H*$_2$CO), 1.90 - 1.83 (1H, m, C*H*CH$_3$), 1.63 (2H, quin, *J* 7.5 Hz, C*H*$_2$CH$_2$CO), 1.58 - 1.41 (3H, m, NHC*H*$_2$C*H*$_2$, CHC*H*$_2$CH$_3$), 1.34 - 1.22 (8H, m, 4 x C*H*$_2$), 1.19 - 1.02 (1H, m, CHC*H*$_2$CH$_3$), 0.94 - 0.44 (12H, m, 4 x C*H*$_3$); δ_H (700 MHz, CD_3OD) 4.20 (1H, d, *J* 7.5 Hz, NHC*H*), 3.21 - 3.06 (2H, m, NHC*H*$_2$CO), 2.26 (2H, t, *J* 7.7 Hz, CH$_2$C*H*$_2$CO), 1.88 - 1.82 (1H, m, C*H*CH$_3$), 1.62 (2H, quin, *J* 7.2 Hz, C*H*$_2$CH$_2$CO), 1.57 - 1.46 (2H, m, C*H*$_2$CH$_2$NH), 1.37 - 1.26 (9H, m, CHC*H*$_2$CH$_3$, 4 x C*H*$_2$), 1.25 - 1.09 (1H, m, CHC*H*$_2$CH$_3$), 0.96 - 0.86 (12H, m, 4 x C*H*$_3$); δ_C (100 MHz, DMSO-d_6) 172.6 (NHCH$_2$*C*O), 170.6 (CH$_2$CH$_2$*C*O), 168.8 (NHCH*C*O), 56.7 (NH*C*HCO), 41.9 (NH*C*H$_2$CO), 40.2 (CH$_3$CH$_2$*C*ONH), 36.8 (*C*HCH$_3$), 35.1 (CH$_2$*C*H$_2$NH), 31.1, 28.5, 26.0, 25.3, 24.3, 22.2 (6 x *C*H$_2$), 15.3, 13.9, 11.4, 11.1 (4 x *C*H$_3$); *m/z* (ESI+) 378.2 ([M+Na]; HR-ESIMS: calculated for $C_{19}H_{37}N_3O_3Na$: 378.2727, found 378.2728 $[M+Na]^+$.

_N_-(Propyl)-(2_S_, 3_S_)-3-methyl-2-(2-octanamidoacetamido)pentaneamide and _N_-(propyl)-(2_R_, 3_S_)-3-methyl-2-(2-octanamidoacetamido)pentaneamide 3.23



Method modified from literature procedure by Crich *et al.*[1] 4 Å Molecular sieves were added to a solution of propylamine (0.09 mL, 1.34 mmol) and acid **3.18** (70: 30

mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR; 0.25 g, 0.80 mmol) in DMF (14 mL), and the mixture was stirred at - 20 °C for 20 minutes. PyBOP (1.0 g, 2.0 mmol) and diisopropylethylamine (0.33 mL, 2.0 mmol) were added and the reaction mixture stirred at - 20 °C for 4 hours. The reaction mixture was filtered, quenched with pH 2 buffer (15 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The combined organics were concentrated *in vacuo*. EtOAc (15 mL) and pH 2 buffer (10 mL) were added and the phases were separated. The organics were washed with pH 2 buffer (10 mL), water (3 x 10 mL), saturated aqueous $NaHCO_3$ solution (10mL) and saturated aqueous NaCl solution (10 mL), dried over Na_2SO_4 and concentrated *in vacuo* (61: 39 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers in crude product, calculated by ^{1}H NMR). The product was obtained by silica column chromatography (10% MeOH: CH_2Cl_2) to give **3.23** as a bright white solid (83 mg, 0.23 mmol, 29 %; 59: 41 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers in crude product, calculated by ^{1}H NMR); υ_{max}/cm^{-1} (neat) 3280 (N-H), 1627 (C=O), 1532 (N-H); δ_H (400 MHz, DMSO-d_6) 8.09 ((2*R,* 3*S*), 1H, t, *J* 6.0 Hz, N*H*CH_2CO), 8.05 ((2*S,* 3*S*), 1H, t, *J* 6.0 Hz, N*H*CH_2CO), 7.98 ((2*S,* 3*S*), 1H, t, *J* 5.5 Hz, N*H*$CH_2CH_2CH_3$), 7.92 ((2*R,* 3*S*), 1H, t, *J* 5.5 Hz, N*H*$CH_2CH_2CH_3$), 7.71 ((2*S,* 3*S*), 1H, d, *J* 9.0 Hz, N*H*CH), 7.66 ((2*R,* 3*S*), 1H, d, *J* 9.0 Hz, N*H*CH), 4.25 ((2*R,* 3*S*), 1H, dd, *J* 9.0, 5.5 Hz, N*H*CH), 4.12 ((2*S,* 3*S*), 1H, dd, *J* 9.0, 7.5 Hz, N*H*CH), 3.81 - 3.61 ((2*S,* 3*S*), 2H, m, NHC*H*$_2$CO; (2*R,* 3*S*), 2H, m, NHC*H*$_2$CO), 3.11 - 2.87 ((2*S,* 3*S*), 2H, m, NHC*H*$_2CH_2CH_3$; (2*R,* 3*S*), 2H, m, NHC*H*$_2CH_2CH_3$), 2.10 ((2*S,* 3*S*), 2H, t, *J* 7.5 Hz, CH_2C*H*$_2$CO; (2*R,* 3*S*), 2H, t, *J* 7.5 Hz, CH_2C*H*$_2$CO), 1.83 - 1.61 ((2*S,* 3*S*), 1H, m, NHCHC*H*$_2$; (2*R,* 3*S*), 1H, m, NHCHC*H*$_2$), 1.55 - 1.33 ((2*S,* 3*S*), 5H, m, NHCHC*H*$_2$, $NHCH_2$C*H*$_2CH_3$, C*H*$_2CH_2$CO; (2*R,* 3*S*), 5H, m, NHCHC*H*$_2$, $NHCH_2$C*H*$_2CH_3$, C*H*$_2CH_2$CO), 1.30 - 1.14 ((2*S,* 3*S*), 8H, m, 4 x C*H*$_2$; (2*R,* 3*S*), 8H, m, 4 x C*H*$_2$), 1.14 - 0.98 ((2*S,* 3*S*), 1H, m, NHCHC*H*$_2$; (2*R,* 3*S*), 1H, m, NHCHC*H*$_2$), 0.91 - 0.72 ((2*S,* 3*S*), 12H, m, CHC*H*$_3$, 4 x C*H*$_3$; (2*R,* 3*S*), 12H, m, CHC*H*$_3$, 4 x C*H*$_3$); δ_H (700 MHz, $CDCl_3$) 7.20 ((2*S*, 3*S*), 1H, d, *J* 8.8 Hz, N*H*CH), 7.13 ((2*R*, 3*S*),1 H, d, *J* 8.8 Hz, N*H*CH), 6.81 ((2*S*, 3*S*), 1H, br. s., N*H*CH_2), 6.78 ((2*R*, 3*S*), 1H,

br. s., N*H*CH_2), 6.61 ((2*S*, 3*S*), 1H, br. s., N*H*CH_2), 6.59 ((2*R*, 3*S*), 1H, br. s., N*H*CH_2), 4.45 ((2*R*, 3*S*), 1H, dd, *J* 8.8, 5.7 Hz, NHC*H*), 4.34 ((2*S*, 3*S*), 1H, t, *J* 7.9 Hz, NHC*H*), 4.12 - 4.04 ((2*S*, 3*S*), 1H, m, NHC*H*$_2$CO; (2*R*, 3*S*), 1H, m, NHC*H*$_2$CO), 4.00 - 3.95 ((2*S*, 3*S*), 1H, m, NHC*H*$_2$CO; (2*R*, 3*S*), 1H, m, NHC*H*$_2$CO), 3.32 - 3.22 ((2*S*, 3*S*), 1H, m, NHC*H*$_2$CH$_2$; (2*R*, 3*S*), 1H, m, NHC*H*$_2$CH$_2$), 3.22 - 3.08 ((2*S*, 3*S*), 1H, m, NHC*H*$_2$CH$_2$; (2*R*, 3*S*), 1H, m, NHC*H*$_2$CH$_2$), 2.26 ((2*S*, 3*S*), 2H, t, *J* 7.7 Hz, CH$_2$C*H*$_2$CO; (2*R*, 3*S*), 2H, t, *J* 7.7 Hz, CH$_2$C*H*$_2$CO), 1.95 - 1.89 ((2*R*, 3*S*), 1H, m, C*H*CH$_3$), 1.89 - 1.82 ((2*S*, 3*S*), 1H, m, C*H*CH$_3$), 1.63 ((2*S*, 3*S*), 2H, quin, *J* 7.5 Hz, C*H*$_2$CH$_2$CO; (2*R*, 3*S*), 2H, quin, *J* 7.5 Hz, C*H*$_2$CH$_2$CO), 1.57 - 1.47 ((2*S*, 3*S*), 3H, m, NHCH$_2$C*H*$_2$, CHC*H*$_2$CH$_3$; (2*R*, 3*S*), 2H, m, NHCH$_2$C*H*$_2$), 1.47 - 1.38 ((2*R*, 3*S*), 3H, m, CHC*H*$_2$CH$_3$), 1.37 - 1.22 ((2*S*, 3*S*), 8H, m, 4 x C*H*$_2$; (2*R*, 3*S*), 8H, m, 4 x C*H*$_2$), 1.22 - 1.06 ((2*S*, 3*S*), 1H, m, CHC*H*$_2$CH$_3$; (2*R*, 3*S*), 1H, m, CHC*H*$_2$CH$_3$), 0.96 - 0.81 ((2*S*, 3*S*), 12H, m, 4 x C*H*$_3$; (2*R*, 3*S*), 12H, m, 4 x C*H*$_3$); δ_H (700 MHz, CD_3OD) 4.35 ((2*R,* 3*S*), 1H, d, *J* 5.7 Hz, NHC*H*), 4.20 ((2*S,* 3*S*), 1H, d, *J* 7.0 Hz, NHC*H*), 3.90 ((2*S*, 3*S*), 1H, d, *J* 16.7 Hz, NHC*H*$_2$CO; (2*R*, 3*S*), 1H, d, *J* 16.7 Hz, NHC*H*$_2$CO), 3.86 ((2*S*, 3*S*), 1H, d, *J* 17.6 Hz, NHC*H*$_2$; (2*R*, 3*S*), 1H, d, *J* 17.6 Hz, NHC*H*$_2$), 3.19 - 3.08 ((2*S*, 3*S*), 2H, m, CH$_2$C*H*$_2$NH; (2*R*, 3*S*), 2H, m, CH$_2$C*H*$_2$NH), 2.26 ((2*S*, 3*S*), 2H, t, *J* 7.7 Hz, CH$_2$C*H*$_2$CO; (2*R*, 3*S*), 2H, t, *J* 7.7 Hz, CH$_2$C*H*$_2$CO), 1.96 - 1.88 ((2*R*, 3*S*), 1H, m, C*H*CH$_3$), 1.87 - 1.79 ((2*S*, 3*S*), 1H, m, C*H*CH$_3$), 1.62 ((2*S*, 3*S*), 2H, quin, *J* 7.0 Hz, C*H*$_2$CH$_2$CO; (2*R*, 3*S*), 2H, quin, *J* 7.0 Hz, C*H*$_2$CH$_2$CO), 1.56 - 1.48 ((2*S*, 3*S*), 2H, m, C*H*$_2$CH$_2$NH; (2*R*, 3*S*), 2H, m, C*H*$_2$CH$_2$NH), 1.43 - 1.27 ((2*S*, 3*S*), 9H, m, CHC*H*$_2$CH$_3$, 4 x C*H*$_2$; (2*R*, 3*S*), 9H, m, CHC*H*$_2$CH$_3$, 4 x C*H*$_2$), 1.25 - 1.12 ((2*S*, 3*S*), 1H, m, CHC*H*$_2$CH$_3$; (2*R*, 3*S*), 1H, m, CHC*H*$_2$CH$_3$), 0.96 - 0.86 ((2*S*, 3*S*), 12H, m, 4 x C*H*$_3$; (2*R*, 3*S*), 12H, m, 4 x C*H*$_3$); δ_C (100 MHz, DMSO-d_6) 172.7, 172.6 ((2*S,* 3*S*), NHCH$_2$*C*O; (2*R,* 3*S*), NHCH$_2$*C*O), 170.7, 170.6 ((2*S,* 3*S*), CH$_2$CH$_2$*C*O; (2*R,* 3*S*), CH$_2$CH$_2$*C*O), 169.1, 168.8 ((2*S,* 3*S*), NHCH*C*O; (2*R,* 3*S*), NHCH*C*O), 56.7 ((2*S,* 3*S*), NH*C*HCO), 55.6 ((2*R,* 3*S*), NH*C*HCO), 42.1, 41.9 ((2*S,* 3*S*), NH*C*H$_2$CO; (2*R,* 3*S*), NH*C*H$_2$CO), 40.3 ((2*S,* 3*S*), CH$_3$CH$_2$*C*H$_2$NH; (2*R,* 3*S*), CH$_3$CH$_2$*C*H$_2$NH), 36.9, 36.8 ((2*S,* 3*S*), *C*HCH$_3$; (2*R,* 3*S*), *C*HCH$_3$), 35.1 ((2*S,* 3*S*), CH$_2$*C*H$_2$CONH; (2*R,* 3*S*),

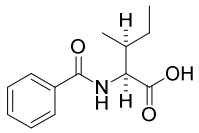
CH_2CH_2CONH), 31.1, 28.6, 28.5, 25.7, 25.2, 24.2, 22.2, 22.1 ((2*S,* 3*S*), 6 x CH_2; (2*R,* 3*S*), 6 x CH_2), 15.3, 14.4, 13.9, 11.5, 11.4, 11.1 ((2*S,* 3*S*), 4 x CH_3; (2*R,* 3*S*), 4 x CH_3); *m/z* (ESI+) 378.1 ([M+Na]; HR-ESIMS: calculated for $C_{19}H_{37}N_3O_3Na$: 378.2727, found 378.2712 $[M+Na]^+$.

_N_-(Propyl)-(2*S*, 3*S*)-3-methyl-2-(2-octanamidoacetamido)pentaneamide and _N_-(propyl)-(2*R*, 3*S*)-3-methyl-2-(2-octanamidoacetamido)pentaneamide 3.21 from thioester

This compound was also prepared from the thioester **3.19**, according to the method of Crich *et al.*[1] A solution of the thioester **3.19** (66 mg, 0.14 mmol, 80: 20 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers) in 40 % piperidine in DMF (1.5 mL) was stirred at room temperature for 1.5 hours. The solution was reduced *in vacuo* and the residue was partitioned between EtOAc (5 mL) and 1M aqueous HCl solution (5 mL). The separated organic phase was washed with water (3 x 5 mL) and saturated aqueous NaCl solution (5 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the crude thioacid, which was used immediately without further purification.

Cs_2CO_3 (53 mg, 0.16 mmol) was added to a solution of the thioacid (0.14 mmol) in DMF (0.25 mL) under a nitrogen atmosphere. After 10 minutes, a solution of the sulfonamide **3.01** (31 mg, 0.11 mmol) in DMF (0.2 mL) was added to give a dark red solution. The reaction mixture was stirred at room temperature for 1 hour, then poured into EtOAc (10 mL). The organics were washed with ice-cold 1M aqueous NaOH solution (5 mL), water (3 x 5mL) and saturated aqueous NaCl solution (5 mL), dried over Na_2SO_4 and concentrated *in vacuo* (73: 27 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers present in crude product, calculated by ^{1}H NMR). Purification by silica chromatography (2% MeOH: CH_2Cl_2 to 10% MeOH: CH_2Cl_2) gave the peptide **3.21** as a yellow oil (21 mg, 0.06 mmol, 43%, 73: 27 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR); All characterisation data was identical to the product prepared previously.

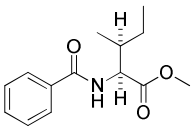
Benzoyl-L-isoleucine 3.24



A solution of benzoyl chloride (2.9 mL, 25 mmol) in THF (30 mL) was added slowly to a solution of L-isoleucine (3.9 g, 30 mmol), potassium carbonate (4.2 g, 30 mmol) and potassium hydroxide (1.7 g, 30 mmol) in water (60 mL) at room temperature to give a cloudy suspension. The reaction was stirred at room temperature overnight. The reaction mixture was acidified to pH 2 with aqueous hydrochloric acid (3M solution) and concentrated *in vacuo*. EtOAc (50 mL) was added and the phases separated. The organic phase was washed with pH 2 buffer (20 mL) and saturated aqueous NaCl solution (20 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the product as a waxy solid (6.81 g, 29.0 mmol, 96 %); m.p. 115 - 116 °C (lit.[11] 135 – 136 °C); $[\alpha]_D^{28}$ +37.7 (c = 0.42, $CHCl_3$), (lit.[12] $[\alpha]_D^{28}$ +31.6 (c = 2.25, $CDCl_3$); υ_{max}/cm^{-1}(neat) 3309 (O-H), 2965 (N-H), 1712 (acid C=O), 1619 (amide C=O), 1533 (N-H); δ_H (400 MHz, $CDCl_3$) 9.81 (1H, br. s., CO_2H), 7.81 (2H, d, *J* 8.5 Hz, 2 x *ortho* Ar C*H*), 7.57 - 7.50 (1H, m, *para* Ar C*H*), 7.50 - 7.39 (2H, m, 2 x *meta* Ar C*H*), 6.75 (1H, d, *J* 8.0 Hz, CHN*H*), 4.86 (1H, dd, *J* 8.5, 5.0 Hz, C*H*NH), 2.17 - 2.06 (1H, m, C*H*CH_3), 1.68 - 1.52 (1H, m, CH_2CH_3), 1.39 - 1.23 (1H, m, CH_2CH_3), 1.03 (3H, d, *J* 6.5 Hz, CHCH_3), 0.98 (3H, t, *J* 7.5 Hz, CH_2CH_3); δ_H (700 MHz, DMSO-d_6) 12.57 (1H, br. s., CO_2H), 8.39 (1H, d, *J* 8.0 Hz, N*H*CH), 7.88 (2H, d, *J* 8.0 Hz, 2 x *ortho* Ar C*H*), 7.54 (1H, t, *J* 7.1 Hz, *para* Ar C*H*), 7.46 (2H, t, *J* 7.7 Hz, 2 x *meta* Ar C*H*), 4.33 (1H, t, *J* 7.5 Hz, NHC*H*), 1.98 - 1.92 (1H, m, C*H*CH_3), 1.55 - 1.49 (1H, m, CHCH_2CH_3), 1.32 - 1.23 (1H, m, CHCH_2CH_3), 0.93 (3H, d, *J* 7.1 Hz, CHCH_3), 0.87 (3H, t, *J* 7.4 Hz, CH_2CH_3); δ_H (700 MHz, CD_3OD) 7.83 (2H, d, *J* 7.2 Hz, 2 x *ortho* Ar C*H*), 7.54 (1H, t, *J* 7.5 Hz, *para* Ar C*H*), 7.47 (2H, t, *J* 7.8 Hz, 2 x *meta* Ar C*H*), 4.56 (1H, d, *J* 6.4 Hz, NHC*H*), 2.06 - 2.01 (1H, m, C*H*CH_3), 1.62 (1H, dqd, *J* 14.5, 7.5, 4.4 Hz, CHCH_2CH_3), 1.38 - 1.31 (1H, m, CHCH_2CH_3), 1.03 (3H, d, *J* 6.9 Hz, CHCH_3), 0.97 (3H, t, *J* 7.5 Hz, CH_2CH_3); δ_C

(100 MHz, $CDCl_3$) 176.0 (*C*O_2H), 167.8 (*C*ONH), 133.8 (*ipso* Ar *C*), 131.9 (*ortho* Ar *CH*), 128.7 (*para* Ar *CH*), 127.1 (*meta* Ar *CH*), 56.9 (*C*HNH), 37.9 (*C*HCH_3), 25.2 (*C*H_2CH_3), 15.5 (CH*C*H_3), 11.6 (CH_2*C*H_3); *m/z* (ESI-) 234.0 ([M-H], 100%); HR-ESIMS: calculated for $C_{13}H_{18}NO_3$: 236.1284, found 236.1281 $[M+H]^+$.The data is consistent with that previously reported.[12]

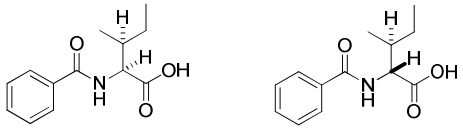
Benzoyl-L-isoleucine 3.25



Acetyl chloride (1.8 mL, 15.5 mmol) was added dropwise to stirred and cooled methanol (10 mL) to give a methanolic solution of HCl. To this was added benzoyl-L-isoleucine **3.24** (1.5 g, 6.4 mmol) and the reaction mixture was heated to reflux for 3 hours. The reaction mixture was concentrated *in vacuo* to give the methyl ester as a white crystalline solid (1.6 g, 6.4 mmol, 99 %); m.p. 88 – 90 °C (lit.[13] 89 °C); $[\alpha]_D^{22}$ +44.3 (c = 1.04 $CHCl_3$), (lit.[14] $[\alpha]_D$+32.0 (c = 10.0 $CHCl_3$)); υ_{max}/cm^{-1}(neat) 3339 (N-H), 1735 (ester C=O), 1638 (amide C=O), 1517 (N-H); δ_H (400 MHz, $CDCl_3$) 7.90 - 7.13 (2H, m, 2 x *ortho* Ar C*H*), 7.45 - 7.40 (1H, m, *para* Ar C*H*), 7.37 - 7.32 (2H, m, 2 x *meta* Ar C*H*), 6.87 (1H, d, *J* 8.0 Hz, N*H*CH), 4.75 (1H, dd, *J* 8.5, 5.5 Hz, NHC*H*), 3.69 (3H, s, OC*H*$_3$), 2.06 - 2.00 (1H, m, C*H*CH_3), 1.48 (1H, dqd, *J* 13.5, 7.5, 4.5 Hz, C*H*$_2CH_3$), 1.22 (1H, ddq, *J* 13.5, 9.0, 7.5 Hz, C*H*$_2CH_3$),0.93 (3H, d, *J* 7.0 Hz, CHC*H*$_3$), 0.91 (3H, t *J* 7.5 Hz, CH_2C*H*$_3$); δ_H (700 MHz, DMSO-d_6) 8.58 (1H, d, *J* 7.7 Hz, N*H*CH), 7.87 (2H, d, *J* 8.3 Hz, 2 x *ortho* Ar C*H*), 7.54 (1H, t, *J* 6.7 Hz, *para* Ar C*H*), 7.47 (2H, t, *J* 8.0 Hz, 2 x *meta* Ar C*H*), 4.35 (1H, t, *J* 7.7 Hz, NHC*H*), 3.65 (3H, s, OC*H*$_3$), 2.01 - 1.93 (1H, m, C*H*CH_3), 1.56 - 1.47 (1H, m, C*H*$_2CH_3$), 1.31 - 1.22 (1H, m, C*H*$_2CH_3$), 0.90 (3H, d, *J* 6.7 Hz, CHC*H*$_3$), 0.87 (3H, t, *J* 7.5 Hz, CH_2C*H*$_3$); δ_H (700 MHz, CD_3OD) 7.83 (2H, dd, *J* 8.3, 1.1 Hz, 2 x *ortho* Ar C*H*), 7.56 - 7.53 (1H, m, *para* Ar C*H*), 7.47 (2H, t, *J* 7.8 Hz, 2 x *meta* Ar C*H*), 4.56 (1H, d, *J* 6.9 Hz, NHC*H*), 3.75 (3H, s, OC*H*$_3$), 2.06 - 1.99 (1H, m, C*H*CH_3), 1.59 (1H, dqd, *J* 13.5, 7.5, 4.2 Hz, C*H*$_2CH_3$), 1.36 - 1.28 (1H, m, C*H*$_2CH_3$), 0.99 (3H, d, *J*

6.7 Hz, CHC*H*$_3$), 0.96 (3H, t, *J* 7.5 Hz, CH$_2$C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 172.4 (*C*O_2CH_3), 167.0 (*C*ONH), 133.8 (*ipso* Ar *C*), 131.4 (*para* Ar *CH*), 128.3 (*meta CH*), 126.9 (*ortho CH*), 56.6 (NH*C*H), 51.9 (CO_2CH_3), 37.8 (*C*HCH$_3$), 25.1 (*C*H$_2$CH$_3$), 15.2 (CH*C*H$_3$), 11.3 (CH$_2$*C*H$_3$); *m/z* (ESI+) 272.0 ([M+Na]+), 100%), 250.0 ([M+H]+), 43%); HR-ESIMS: calculated for $C_{14}H_{20}NO_3$: 250.1438, found 250.1439 $[M+H]^+$. ^{1}H NMR spectroscopic data is consistent with that previously reported.[14]

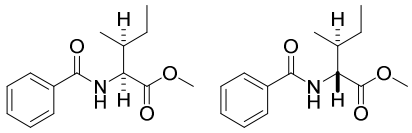
Benzoyl-L-isoleucine and benzoyl-D-*allo*-isoleucine 3.26



Prepared as for the single diastereomer, using a mixture of L- and D-*allo*-isoleucine (0.50 g, 3.8 mmol) to give the product as a waxy yellow solid (0.81 g, 3.4 mmol, 90 %, 78: 22 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR); υ_{max} /cm^{-1}(neat) 3346 (O-H), 2874 (N-H), 1720 (acid C=O), 1628 (amide C=O), 1535 (N-H); δ_H (400 MHz, $CDCl_3$) 9.57 ((2*S,* 3*S*), 1H, br.s., CO_2H; (2*R,* 3*S*), 1H, br.s., CO_2H), 7.81 ((2*S*, 3*S*), 2H, d, *J* 8.0 Hz, 2 x *ortho* Ar C*H*; (2*R,* 3*S*), 2H, d, *J* 8.0 Hz, 2 x *ortho* Ar C*H*), 7.56 - 7.49 ((2*S,* 3*S*), 1H, m, *para* Ar C*H*; (2*R,* 3*S*), 1H, m, *para* Ar C*H*), 7.48 - 7.42 ((2*S,* 3*S*), 2H, m, 2 x *meta* Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x *meta* Ar C*H*), 6.74 ((2*S,* 3*S*), 1H, d, *J* 8.0 Hz, CHN*H*), 6.66 ((2*R,* 3*S*), 1H, d, *J* 9.0 Hz, CHN*H*), 4.97 ((2*R,* 3*S*), 1H, dd, *J* 9.0, 4.0 Hz, C*H*NH), 4.85 ((2*S,* 3*S*), 1H, dd, *J* 8.5, 5.0 Hz, CHN*H*), 2.23 - 2.02 ((2*S,* 3*S*), 1H, m, C*H*CH$_3$; (2*R,* 3*S*), 1H, m, C*H*CH$_3$), 1.69 - 1.47 ((2*S,* 3*S*), 1H, m, C*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, C*H*$_2$CH$_3$), 1.41 - 1.21 ((2*S,* 3*S*), 1H, m, C*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, C*H*$_2$CH$_3$), 1.03 ((2*S,* 3*S*), 3H, d, *J* 6.5 Hz, CHC*H*$_3$; (2*R,* 3*S*), 3H, d, *J* 6.5 Hz, CHC*H*$_3$), 1.05 - 0.95 ((2*R,* 3*S*), 3H, m, CH$_2$C*H*$_3$; (2*S,* 3*S*), 3H, m, CH$_2$C*H*$_3$); δ_H (700 MHz, DMSO-d_6) 12.57 ((2*S,* 3*S*), 1H, br. s., CO_2H; (2*R,* 3*S*), 1H, br. s., CO_2H), 8.39 ((2*S,* 3*S*), 1H, d, *J* 7.7 Hz, N*H*CH), 8.28 ((2*R,* 3*S*), 1H, d, *J* 8.3 Hz, N*H*CH), 7.88 ((2*S*, 3*S*), 2H, d, *J* 8.0 Hz, 2 x *ortho* Ar C*H*; (2*R,* 3*S*), 2H, d, *J* 8.0 Hz, 2 x *ortho* Ar C*H*), 7.54 ((2*S*, 3*S*), 1H, t, *J* 7.4 Hz, *para* Ar

C*H*; (2*R*, 3*S*), 1H, t, *J* 7.4 Hz, *para* Ar C*H*), 7.47 ((2*S*, 3*S*), 2H, t, *J* 7.4 Hz, 2 x *meta* Ar C*H*; (2*R*, 3*S*), 2H, t, *J* 7.4 Hz, 2 x *meta* Ar C*H*), 4.53 ((2*R*, 3*S*), 1H, dd, *J* 8.3, 5.8 Hz, NHC*H*), 4.33 ((2*S*, 3*S*), 1H, t, *J* 7.5 Hz, NHC*H*), 2.03 - 1.98 ((2*R*, 3*S*), 1H, m, C*H*CH_3), 1.98 - 1.92 ((2*S*, 3*S*), 1H, m, C*H*CH_3), 1.56 - 1.48 ((2*S*, 3*S*), 1H, m, C$H_2$$CH_3$), 1.47 - 1.38 ((2*R*, 3*S*), 1H, m, C$H_2$$CH_3$), 1.32 - 1.24 ((2*S*, 3*S*), 1H, m, C$H_2$$CH_3$), 1.24 - 1.16 ((2*R*, 3*S*), 1H, m, C$H_2$$CH_3$), 0.95 ((2*R*, 3*S*), 3H, d, *J* 7.1 Hz, CHCH_3), 0.94 ((2*S*, 3*S*), 3H, d, *J* 6.7 Hz, CHCH_3), 0.91 - 0.86 ((2*S*, 3*S*), 3H, m, CH_2CH_3; (2*R*, 3*S*), 3H, m, CH_2CH_3); δ_H (700 MHz, CD_3OD) 7.85 - 7.82 ((2*S*, 3*S*), 2H, m, 2 x *ortho* Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x *ortho* Ar C*H*), 7.57 - 7.52 ((2*S*, 3*S*), 1H, m, *para* Ar C*H*; (2*R,* 3*S*), 1H, m, *para* Ar C*H*), 7.45 - 7.49 ((2*S*, 3*S*), 2H, m, 2 x *meta* Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x *meta* Ar C*H*), 4.74 ((2*R,* 3*S*), 1H, d, *J* 4.99 Hz, NHC*H*), 4.56 ((2*S,* 3*S*), 1H, d, *J* 6.38 Hz, NHC*H*), 2.13 - 2.07 ((2*R,* 3*S*), 1H, m, C*H*CH_3), 2.07 - 2.01 ((2*S,* 3*S*), 1H, m, C*H*CH_3), 1.62 ((2*S,* 3*S*), 1H, dqd, *J* 14.7, 7.5, 4.4 Hz, C$H_2$$CH_3$), 1.57 - 1.50 ((2*R,* 3*S*), 1H, m, C$H_2$$CH_3$), 1.39 - 1.28 ((2*S,* 3*S*), 1H, m, C$H_2$$CH_3$; (2*R,* 3*S*), 1H, m, C$H_2$$CH_3$), 1.05 - 0.95 ((2*S,* 3*S*), 6H, m, CHCH_3, CH_2CH_3; (2*R,* 3*S*), 6H, m, CHCH_3, CH_2CH_3); δ_C (100 MHz, $CDCl_3$) 176.6, 176.0 ((2*S,* 3*S*), *C*O_2H; (2*R,* 3*S*), *C*O_2H), 167.9, 167.7 ((2*S,* 3*S*), *C*ONH; (2*R,* 3*S*), *C*ONH), 133.8 ((2*S*, 3*S*), *ipso* Ar *C*; (2*R,* 3*S*), *ipso* Ar *C*), 131.9 ((2*S,* 3*S*), 2 x *ortho* Ar *CH*; (2*R,* 3*S*), 2 x *ortho* Ar *CH*), 128.7 ((2*S,* 3*S*), *para* Ar *CH*; (2*R,* 3*S*), *para* Ar *CH*), 127.1 ((2*S,* 3*S*), 2 x *meta* Ar *CH*; (2*R,* 3*S*), 2 x *meta* Ar *CH*), 56.9, 55.7 ((2*S,* 3*S*), *C*HNH; (2*R,* 3*S*), *C*HNH), 38.9, 37.8 ((2*S,* 3*S*), *C*HCH_3; (2*R,* 3*S*), *C*HCH_3), 26.3, 25.2 ((2*S,* 3*S*), *C*$H_2$$CH_3$; (2*R,* 3*S*), *C*$H_2$$CH_3$), 15.5, 14.7 ((2*S,* 3*S*), CH*C*H_3; (2*R,* 3*S*), CH*C*H_3), 11.7, 11.6 ((2*S,* 3*S*), CH_2*C*H_3; (2*R,* 3*S*), CH_2*C*H_3); *m/z* (ESI+) 258.0 ([M+Na]+), 100%); HR-ESIMS: calculated for $C_{13}H_{18}NO_3$: 236.1284, found 236.1281 $[M+H]^+$.This compound as a mix of diastereomers has not been previously reported.

Benzoyl-L-isoleucine methyl ester and benzoyl-D-*allo*-isoleucine methyl ester 3.26



Prepared as for the single diastereomer, using a mixture of L-isoleucine and D-*allo*-isoleucine to give the methyl ester as a white crystalline solid (0.18 g, 7.2 mmol, 85 %, 50:50 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers, calculated by ^{1}H NMR); υ_{max}/cm^{-1}(neat) 3340 (N-H), 1736 (ester C=O) 1638, (amide C=O), 1517 (N-H); δ_H (400 MHz, $CDCl_3$) 7.81 ((2*S,* 3*S*), 2H, d, *J* 7.5 Hz, 2 x *ortho* Ar C*H*; (2*R,* 3*S*), 2H, d, *J* 7.5 Hz, 2 x *ortho* Ar C*H*), 7.58 - 7.49 ((2*S,* 3*S*), 1H, m, *para* Ar C*H*; (2*R,* 3*S*), 1H, m, *para* Ar C*H*), 7.48 - 7.41 ((2*S,* 3*S*), 2H, m, 2 x *meta* Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x *meta* Ar C*H*), 6.70 ((2*S,* 3*S*), 1H, d, *J* 8.0 Hz, CHN*H*), 6.62 ((2*R,* 3*S*), 1H, d, *J* 8.5 Hz, CHN*H*), 4.93 ((2*R,* 3*S*), 1H, dd, *J* 9.0, 4.0 Hz, C*H*NH), 4.82 ((2*S,* 3*S*), 1H, dd, *J* 8.5, 5.0 Hz, C*H*NH), 3.77 ((2*S,* 3*S*), 3H, s, OC*H*$_3$; (2*R,* 3*S*), 3H, s, OC*H*$_3$), 2.11 - 1.95 ((2*S,* 3*S*), 1H, m, C*H*CH_3; (2*R,* 3*S*), 1H, m, C*H*CH_3), 1.61 - 1.44 ((2*S,* 3*S*), 1H, m, C*H*$_2CH_3$; (2*R,* 3*S*), 1H, m, C*H*$_2CH_3$), 1.35 - 1.16 ((2*S,* 3*S*), 1H, m, C*H*$_2CH_3$; (2*R,* 3*S*), 1H, m, C*H*$_2CH_3$), 1.01 - 0.93 ((2*S,* 3*S*), 6H, m, CH_2C*H*$_3$, CHC*H*$_3$; (2*R,* 3*S*), 6H, m, CH_2C*H*$_3$, CHC*H*$_3$); δ_H (700 MHz, DMSO-d_6) 8.58 ((2*S,* 3*S*), 1H, d, *J* 7.7 Hz, N*H*CH), 8.48 ((2*R,* 3*S*), 1H, d, *J* 8.0 Hz, N*H*CH), 7.87 ((2*S,* 3*S*), 2H, d, *J* 7.7 Hz, 2 x *ortho* Ar C*H*; (2*R,* 3*S*), 2H, d, *J* 7.7 Hz, 2 x *ortho* Ar C*H*), 7.55 ((2*S,* 3*S*), 1H, t, *J* 7.1 Hz, *para* Ar C*H*; (2*R,* 3*S*), 1H, t, *J* 7.1 Hz, *para* Ar C*H*), 7.50 - 7.44 ((2*S,* 3*S*), 2H, m, 2 x *meta* Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x *meta* Ar C*H*), 4.54 ((2*R,* 3*S*), 1H, dd, *J* 8.0, 6.4 Hz, NHC*H*), 4.35 ((2*S,* 3*S*), 1H, t, *J* 7.7 Hz, NHC*H*), 3.66 ((2*R,* 3*S*), 3H, s, OC*H*$_3$), 3.65 ((2*S,* 3*S*), 3H, s, OC*H*$_3$), 2.04 - 1.94 ((2*S,* 3*S*), 1H, m, C*H*CH_3; (2*R,* 3*S*), 1H, m, C*H*CH_3), 1.52 ((2*S,* 3*S*), 1H, dqd, *J* 13.9, 7.1, 4.0 Hz, C*H*$_2CH_3$), 1.41 ((2*R,* 3*S*), 1H, dquin, *J* 13.9, 6.9 Hz, C*H*$_2CH_3$), 1.31 - 1.24 ((2*S,* 3*S*), 1H, m, C*H*$_2CH_3$), 1.19 ((2*R,* 3*S*), 1H, dquin, *J* 14.1, 7.3 Hz, C*H*$_2CH_3$), 0.95 ((2*R,* 3*S*), 3H, d, *J* 6.7 Hz, CHC*H*$_3$), 0.92 - 0.85 ((2*S,* 3*S*), 6H, CHC*H*$_3$, CH_2C*H*$_3$; (2*R,* 3*S*), 3H, CH_2C*H*$_3$); δ_H

(700 MHz, CD_3OD) 7.84 - 7.81 ((2*S,* 3*S*), 2H, m, 2 x *ortho* Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x *ortho* Ar C*H*), 7.57 - 7.53 ((2*S,* 3*S*), 1H, m, *para* Ar C*H*; (2*R,* 3*S*), 1H, m, *para* Ar C*H*), 7.50 - 7.44 ((2*S,* 3*S*), 2H, m, 2 x *meta* Ar C*H*; (2*R,* 3*S*), 2H, m, 2 x *meta* Ar C*H*), 4.73 ((2*R,* 3*S*), 1H, d, *J* 5.5 Hz, NHC*H*), 4.56 ((2*S,* 3*S*), 1H, d, *J* 6.9 Hz, NHC*H*), 3.76 ((2*R,* 3*S*), 3H, s, OCH_3), 3.75 ((2*S,* 3*S*), 3H, s, OCH_3), 2.11 - 2.05 ((2*R,* 3*S*), 1H, m, C*H*CH_3), 2.05 - 1.98 ((2*S,* 3*S*), 1H, m, C*H*CH_3), 1.59 ((2*S,* 3*S*), 1H, dqd, *J* 13.5, 7.7, 4.4 Hz, CH_2CH_3), 1.53 - 1.46 ((2*R,* 3*S*), 1H, m, CH_2CH_3), 1.36 - 1.21 ((2*S,* 3*S*), 1H, m, CH_2CH_3; (2*R,* 3*S*), 1H, m, CH_2CH_3), 1.01 - 0.93 ((2*S,* 3*S*), 6H, m, CHCH_3, CH_2CH_3; (2*R,* 3*S*), 6H, m, CHCH_3, CH_2CH_3) δ_C (100 MHz, $CDCl_3$) 172.9, 172.6 ((2*S,* 3*S*), *C*O_2CH_3; (2*R,* 3*S*) *C*O_2CH_3), 167.3, 167.1 ((2*S,* 3*S*), *C*ONH; (2*R,* 3*S*), *C*ONH); 134.1 ((2*S,* 3*S*), *ipso* Ar *C*; (2*R,* 3*S*), *ipso* Ar *C*), 131.7 ((2*S,* 3*S*), *para* Ar *CH*; (2*R,* 3*S*), *para* Ar *CH*), 128.6 ((2*S,* 3*S*), *meta* Ar *CH*; (2*R,* 3*S*), *meta* Ar *CH*), 127.0 ((2*S,* 3*S*), *ortho* Ar *CH*; (2*R,* 3*S*), *ortho* Ar *CH*), 56.7, 55.7 ((2*S,* 3*S*), *C*HNH; (2*R,* 3*S*), *C*HNH), 52.3, 52.1 ((2*S,* 3*S*), CO_2CH_3; (2*R,* 3*S*), CO_2CH_3), 38.1 ((2*S,* 3*S*), *C*HCH_3; (2*R,* 3*S*), *C*HCH_3), 26.3, 25.3 ((2*S,* 3*S*), *C*H_2CH_3; (2*R,* 3*S*), *C*H_2CH_3), 15.4, 14.7 ((2*S,* 3*S*), CH*C*H_3; (2*R,* 3*S*), CH*C*H_3), 11.7, 11.5 ((2*S,* 3*S*), CH_2CH_3; (2*R,* 3*S*), CH_2CH_3); *m/z* (ESI+) 272.1 ([M+Na]+), 100%), 250.1 ([M+Na]+), 32%); HR-ESIMS: calculated for $C_{14}H_{19}NO_3Na$: 272.1257, found 272.1257 $[M+H]^+$. ^{1}H NMR spectroscopic data is consistent with that previously reported.[14]

3.9 EXPERIMENTAL REFERENCES

1. D. Crich, K. Sana and S. Guo, *Org. Lett.*, 2007, **9**, 4423-4426.
2. S. Balamurugan and P. Kannan, *J. Mol. Struct.*, 2009, **934**, 44-52.
3. A. Leydet, H. El Hachemi, B. Boyer, G. Lamaty, J. P. Roque, D. Schols, R. Snoeck, G. Andrei, S. Ikeda, J. Neyts, D. Reymen, J. Este, M. Witvrouw and E. De Clercq, *J. Med. Chem.*, 1996, **39**, 1626-1634.
4. V. du Vigneaud and C. E. Meyer, *J. Biol. Chem.*, 1932, **98**, 295-308.

5. F. Albericio, N. Kneib-Cordonier, S. Biancalana, L. Gera, R. I. Masada, D. Hudson and G. Barany, *J. Org. Chem.*, 1990, **55**, 3730-3743.

6. F. Albericio, E. Nicolas, J. Rizo, M. Ruiz-Gayo, E. Pedroso and E. Giralt, *Synthesis*, 1990, 119 - 122.

7. S. Yamada, C. Hongo, R. Yoshioka and I. Chibata, *J. Org. Chem.*, 1983, **48**, 843-846.

8. G.-h. Tao, L. He, N. Sun and Y. Kou, *Chem. Commun. (Cambridge, U. K.)*, 2005, 3562-3564.

9. N. R. McIntyre, E. W. Lowe, J. L. Belof, M. Ivkovic, J. Shafer, B. Space and D. J. Merkler, *J. Am. Chem. Soc.*, 2010, **132**, 16393-16402.

10. M. Nechab, L. El Blidi, N. Vanthuyne, S. Gastaldi, M. P. Bertrand and G. Gil, *Org. Biomol. Chem.*, 2008, **6**, 3917-3920.

11. B. S. Jursic and D. Neumann, *Synth. Commun.*, 2001, **31**, 555-564.

12. S. Huneck, A. Porzel and J. Schmidt, *Tetrahedron: Asymmetry*, 1993, **4**, 303-311.

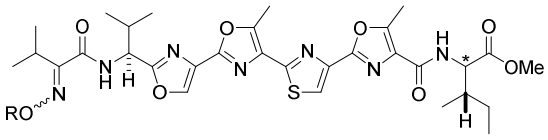
13. D. T. S. Rijkers, H. P. H. M. Adams, H. C. Hemker and G. I. Tesser, *Tetrahedron*, 1995, **51**, 11235-11250.

14. I. É. Khatskevich, I. K. Kalnin, E. I. Karpeiskaya and E. I. Klabunovskii, *Bull. Acad. Sci. USSR, Div. Chem. Sci. (Engl. Transl.)*, 1983, **32**, 323-329.

CHAPTER 4: THE TOTAL SYNTHESIS OF THE AZOLEMYCINS AND RELATED COMPOUNDS

4.1 AZOLEMYCIN

The azolemycins are a group of compounds recently isolated from the soil bacterium, *Streptomyces* sp. FXJ1.264. Professor Challis and Dr Song (Department of Chemistry, University of Warwick) used a combination of mass spectrometry and 1D and 2D NMR to identify the structure as a modified heptapeptide, with four linked azoles and an *N*-terminal oxime. The major isolated azolemycin is the *N*-hydroxy derivative, azolemycin A, **4.01**, while the minor, azolemycin B, **4.02**, has an *N*-methoxy group (Figure 34). There is currently some doubt as to whether azolemycin B is actually a microbially derived peptide, or a relic of the purification techniques used.



4.01 Azolemycin A, R = H
4.02 Azolemycin B, R = Me

Figure 34: The structures of azolemycins A and B

The bacterial genome of *Streptomyces* sp. FXJ1.264. has been sequenced, so it has been possible to identify the associated genes involved in the biosynthesis of azolemycin A, based on the peptide sequence VVSTCTI. It is proposed that a ribosomally synthesised peptide precursor containing the seven amino-acid sequence is first dehydrated and then oxidised to give the tetra-azole sequence. The surrounding amino-acids are then cleaved, and the *N*-terminal amine is oxidised to give the oxime, and the isoleucine methyl ester is formed (Figure 35). Oxidation of an amine to an oxime is unusual in natural products and, as far as we know, this is an unprecedented reaction for the enzyme responsible for the azolemycin oxime formation, a flavin dependent mono-oxygenase.

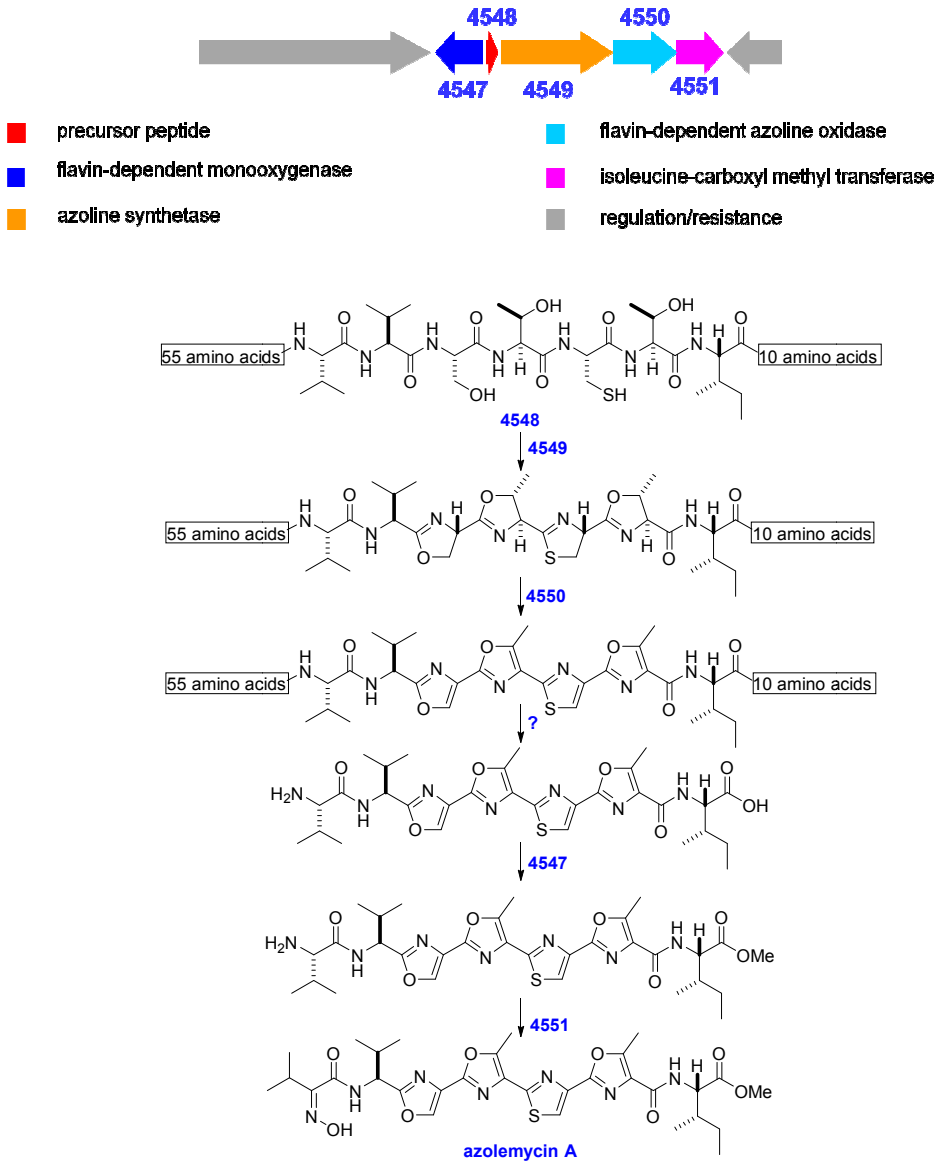


Figure 35: The biosynthesis of azolemycin A in *Streptomyces* sp. FXJ1.264 and the associated gene clusters proposed by Professor Challis (University of Warwick)

It has been proposed that this flavin dependent *N*-oxidation could proceed *via* the *N*-hydroxylamine and nitroso- intermediates. Similar mechanisms are known in the literature, including the oxidation of an amine to an oxime in the biosynthesis of norcardicin A, a β-lactam antibiotic derived from an actinomycete (Figure 36).[1] However, this oxidation is controlled by a heme-dependent cytochrome P450 enzyme. Reported reactions for non-heme dependent enzymes go straight to the nitro compounds, as in the biosynthesis of pyrollnitrin.[2, 3] We believe that the enzyme involved in the biosynthesis of azolemycin A follows a similar mechanism to the

cytochrome P450 reaction, stopping at the oxime, but utilises a non-heme dependent enzyme as seen in the biosynthesis of pyrollnitrin.

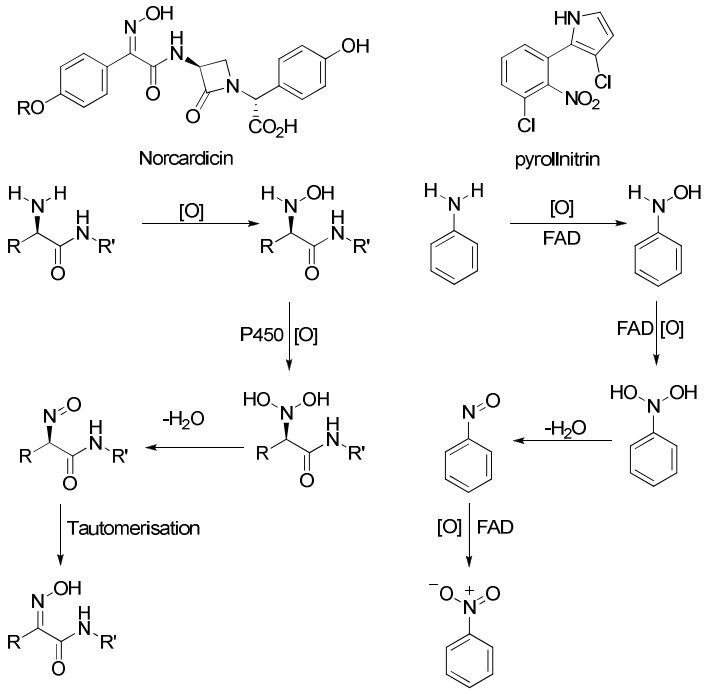


Figure 36: The two oxidation pathways proposed for the biosynthesis of Norcardicin and pyrollnitrin

4.2 ASSIGNMENT OF STEREOCHEMISTRY OF *C*-TERMINAL ISOLEUCINE

4.2.1 USE OF BENZOYL-ISOLEUCINE MODEL

At the time we became involved in the project, the amino-acid sequence and structure of azolemycin A had been solved, but there was some ambiguity over the stereochemistry of the *C*-terminal isoleucine. We realised that the benzoyl-isoleucine methyl ester diastereomers we had previously synthesized, **3.25** and **3.27**, (Scheme 22) could act as a model for the last two *C*-terminal residues. We compared the ^{1}H NMR spectrum for the mix of benzoyl-L-isoleucine methyl ester and benzoyl-D-*allo*-isoleucine methyl ester with that of azolemycin A (Figure 37, Figure 38). The signal associated with the isoleucine α-C<u>H</u> of azolemycin occurred at a lower field than either of the diastereomers of benzoyl-isoleucine methyl ester, but the chemical shift and coupling constant led us to tentatively assign the stereochemistry as that of L-isoleucine rather than that of D-*allo*-isoleucine.

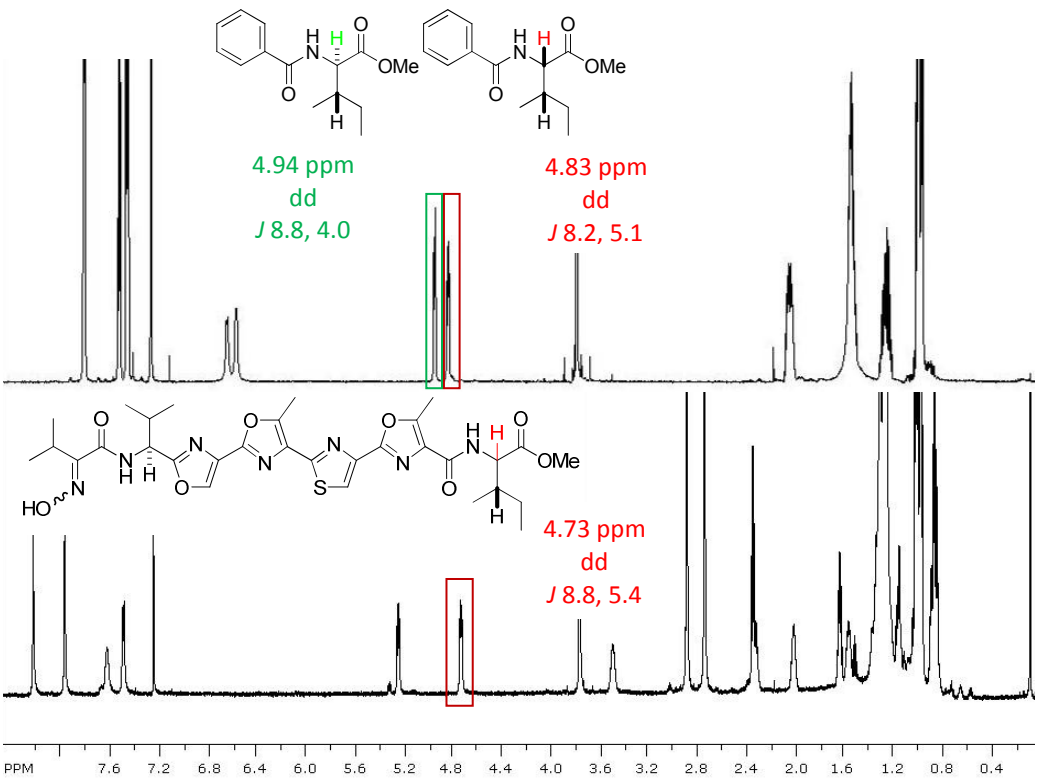


Figure 37: The ^{1}H NMR spectra for the mix of diastereomers of benzoyl-isoleucine methyl esters (top spectrum) and for azolemycin A (bottom spectrum), with the signals associated with the α-CH highlighted

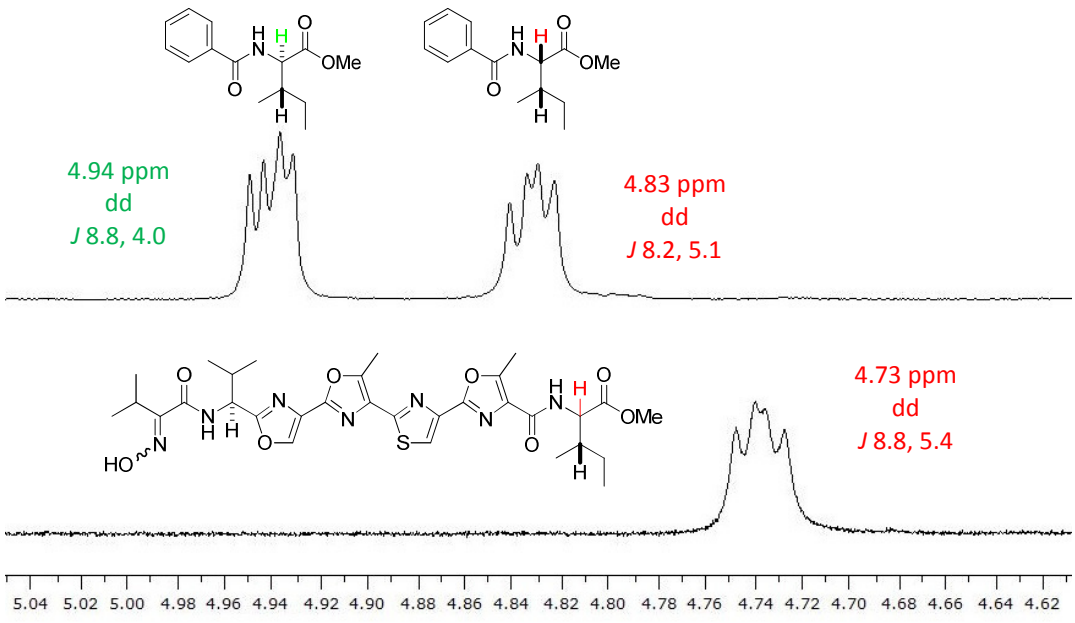


Figure 38: Highlighting the signals associated with the α-CH for mix of diastereomers of benzoyl-isoleucine methyl esters (top) and azolemycin (bottom)

4.2.2 RETROSYNTHESIS OF THE OXAZOLE MODEL

In order to reinforce this stereochemical assignment, we decided to synthesise an extended analogue, 5-methyl-2-phenyl-oxazole-4-carboxy-isoleucine methyl ester, (Figure 39), again as both the L-isoleucine diastereomer **4.06**, and the mix of L- and D-*allo*-isoleucine diastereomers, **4.07**. This would hopefully provide a more accurate model for the *C*-terminus of azolemycin A.

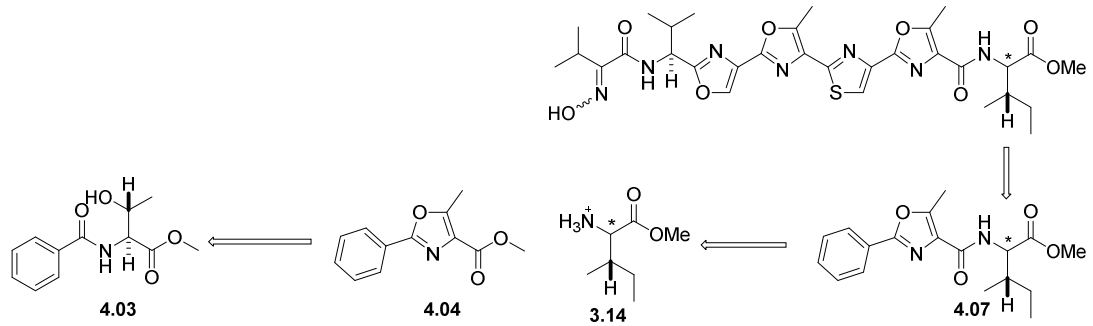


Figure 39: Proposed mimic for the last three *C*-terminal residues of azolemycin A, and the retrosynthesis to the phenyl oxazole , **4.04**, and isoleucine methyl ester, **3.14**

4.3 SYNTHESIS OF OXAZOLES

Oxazoles, particularly the 3, 5-substituted oxazoles seen in azolemycin, are relatively common in natural products,[4] and a number of methods have been developed for their synthesis.[5, 6] When the starting point is a natural amino-acid (here benzoyl-L-threonine methyl ester, **4.03**, the general route involves a dehydration followed by an oxidation (Figure 40, **A**), or an oxidation followed by dehydration (Figure 39, **B**). While this limits the substituents at the 2- position to either a hydrogen or a methyl group, it has the advantage of simplicity, and does allow a certain range of substituents at the 3- and 5- positions.

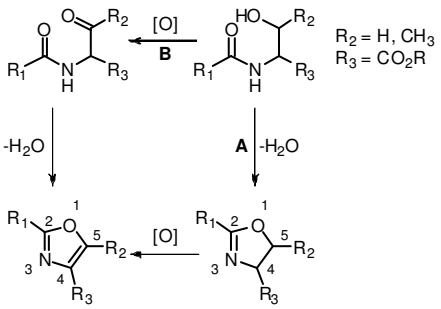


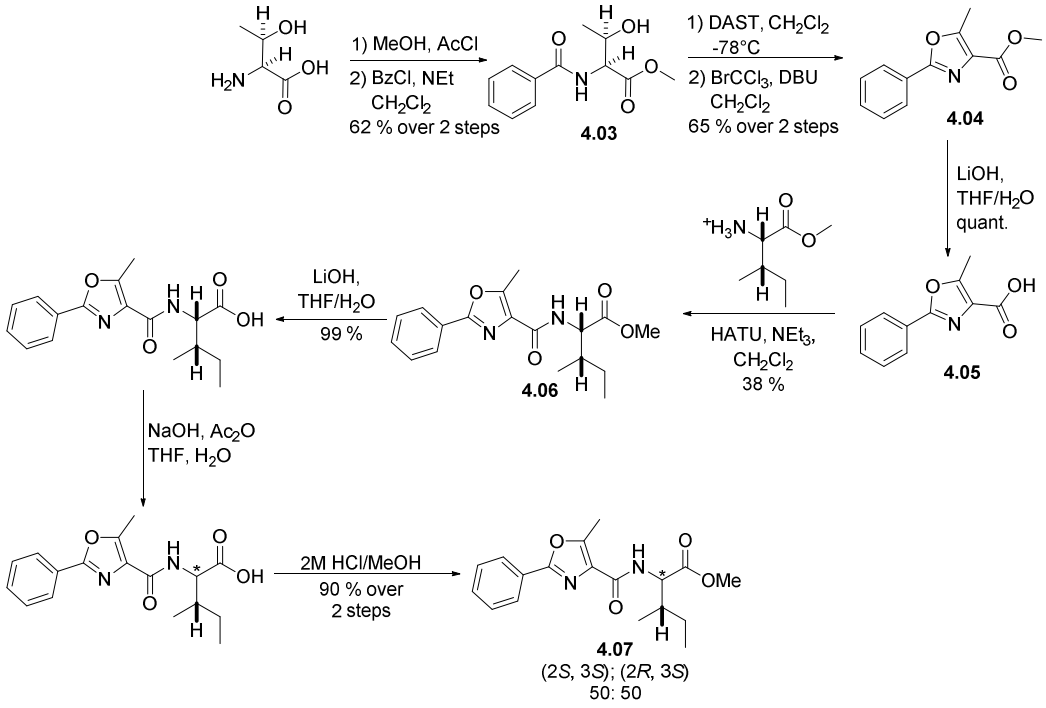
Figure 40: A general route to oxazoles from *C*- and *N*-substituted serine or threonine.

In the case of route **A,** a range of dehydrating agents have been utilised to form the oxazoline intermediate, including thionyl chloride,[7] sulfonyl chlorides,[8-10] PPh_3 with DEAD or CCl_4,[10, 11] Burgess reagent,[12-14] and the fluorinating agents DAST and Deoxo-Fluor.[15] A similarly wide range of oxidation agents have been reported to produce the oxazole, including $BrCCl_3$ and DBU,[16] $CuBr_2$,[17] MnO_2,[18] DDQ,[19] and NiO_2.[20]

In route **B**, the initial oxidation is generally performed using Parikh-Doering conditions,[21, 22] Dess-Martin periodinane,[23] Jones reagent,[24] or similar, before a Robinson-Gabriel type dehydration is performed to give the oxazole.[25, 26] Again, a wide variety of dehydrating agents have been reported, but most commonly these include PPh_3-I_2-NEt_3,[27] thionyl chloride,[28] H_2SO_4,[29] $POCl_3$,[30] and P_2O_5.[31]

Considering the gamut of reagents available for these two, relatively simple, routes, it was obvious that the ideal combination could depend on the particular substrate used. We planned to synthesise the phenyl oxazole **4.04** using the amino-acid precursor, benzoyl-L-threonine methyl ester **4.03**. Having considered the literature, we successfully used DAST for the initial oxazoline formation, followed by a $BrCCl_3$-DBU oxidation to give the phenyl oxazole methyl ester (Scheme 23), as described by Pattenden *et al*. in their synthesis of the polyoxazole cyclopeptide YM-216391 (Figure 46).[32]

The methyl ester was hydrolysed and a peptide coupling reaction gave the 5-methyl-2-phenyl-oxazole-4-carboxy-L-isoleucine methyl ester, **4.06**. A portion of this was hydrolysed to the carboxylic acid and epimerised using the method previously described (Chapter 3, Scheme 17). The methyl ester was reformed to give the 5-methyl-2-phenyl-oxazole-4-carboxy-isoleucine methyl ester as a 1: 1 mix of L-isoleucine and D-*allo*-isoleucine diastereomers, **4.07**. The ^{1}H NMR spectra of **4.06** and **4.07** were compared to confirm the shifts associated with the α-C<u>H</u> of the two diastereomers.



Scheme 23: The route to the 5-methyl-2-phenyl-oxazole-4-carboxy-isoleucine methyl ester as both the single L-isoleucine diastereomer, **4.06**, and the mix of L- and D-*allo*-isoleucine diastereomers, **4.07**

Comparison of the ^{1}H NMR spectra of the mix of diastereomers and azolemycin A showed a near complete overlap between the peak associated with the α-C<u>H</u> of the isoleucine in azolemycin A and the peak of the L-diasteromer of 5-methyl-2-phenyl-oxazole-4-carboxy-isoleucine methyl ester (within 0.01 ppm), and similarly close coupling constants (Figure 41, Figure 42). This reinforced our tentative assignment of the relative stereochemistry of the isoleucine residue in azolemycin A as either L-isoleucine or D-isoleucine, with L-isoleucine being the most likely enantiomer.

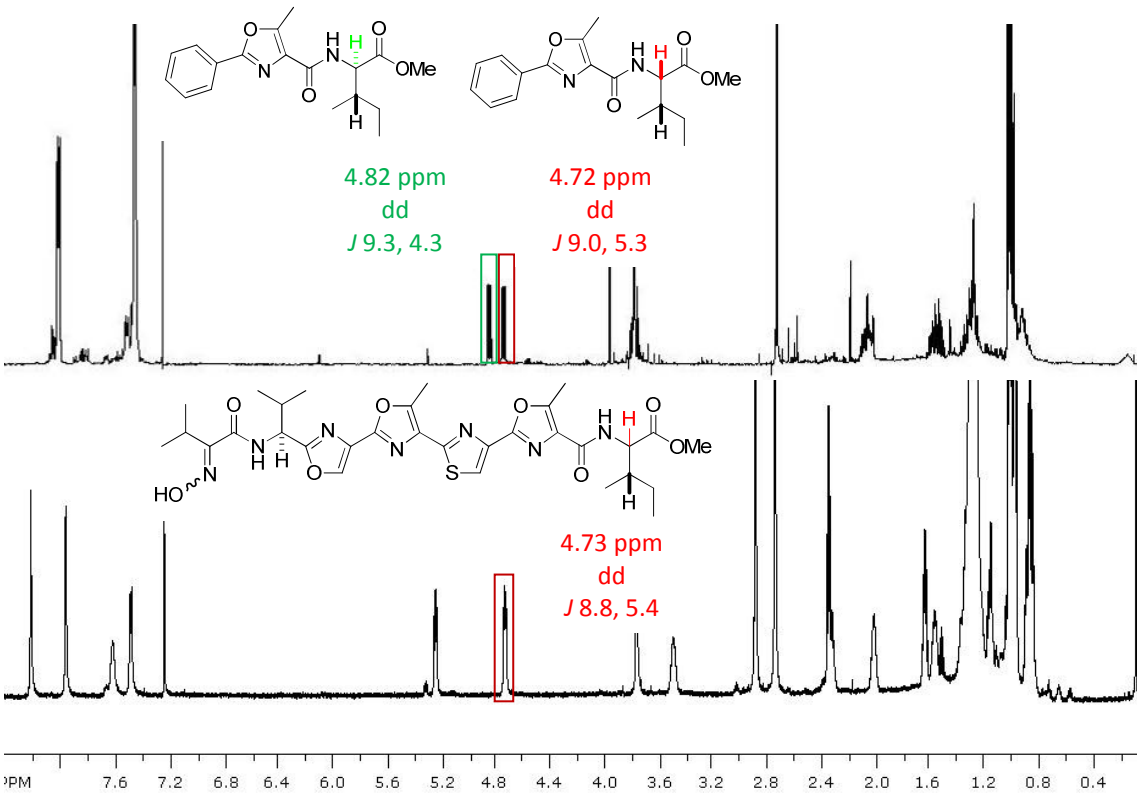


Figure 41: The ^{1}H NMR spectra for the mix of diastereomers, **4.07**, (top spectrum) and for azolemycin A **4.01**(bottom spectrum), with the signals associated with the α-CH highlighted

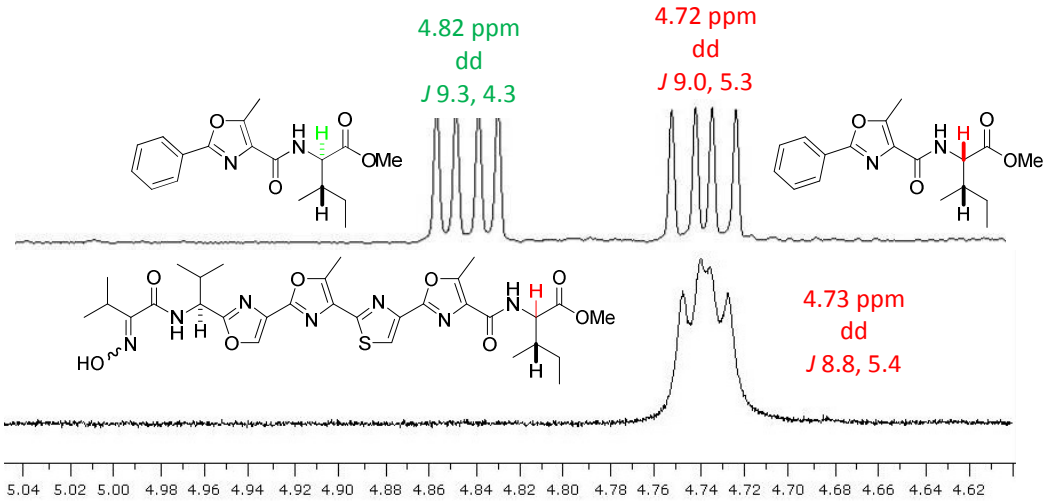


Figure 42: Highlighting the signals associated with the α-CH for mix of diastereomers of benzoyl-isoleucine methyl esters, **4.07**, (top), and azolemycin A, **4.01** (bottom)

The stereochemistry of the terminal isoleucine was later confirmed by Marfeys' analysis of the natural product, and it was extremely pleasing to see our hypothesis proved in the characterisation of a new natural product.

4.4 THE TOTAL SYNTHESIS OF POLYAZOLE NATURAL PRODUCTS

With the successful synthesis of the model oxazole **4.07**, our interest in the natural product, azolemycin A, increased. At the time, only 1 mg of the product had been extracted and it was from this that the structure had been obtained and the stereochemistry confirmed. Azolemycin A is a relatively simple natural product, and we realised it could be entirely synthesised from naturally occurring amino-acids. In addition, it has only two stereocentres that would be at risk of epimerisation, and we were confident that the experience we had gained in peptide coupling would allow us to complete a total synthesis of azolemycin A without risking epimerisation of the valine and isoleucine α-C<u>H</u> centres.

Until the 1980's, very few thiazole and oxazole-containing natural products had been known, but an increasing array have since been identified, particularly derived from marine sources.[5, 6, 33-35] These products range from single oxazoles and thiazoles to more complicated linked chains or macrocycles of concatenated azoles. The combination of potent biological activity of many of these azole products and the exciting synthetic challenges they pose has made them extremely attractive targets for total syntheses.[4, 36, 37]

Polyazoles, or polyazole-containing, natural products tackled in total syntheses range from the relatively simple linked bis- and triazole motifs, as found in the hennoxazoles,[38] muscorides (Figure 43),[39, 40] bleomycins,[41, 42] and myxothiazols,[43, 44] to more complicated macrocyclic molecules such as the heptazole telemestatin and the related YM-216391.

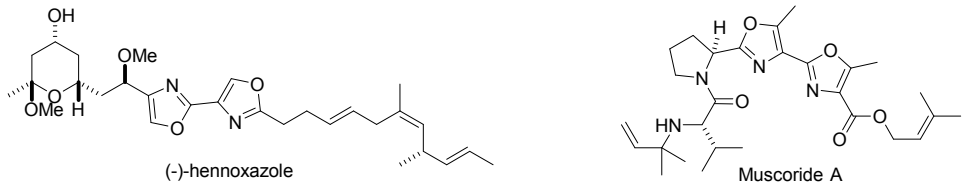
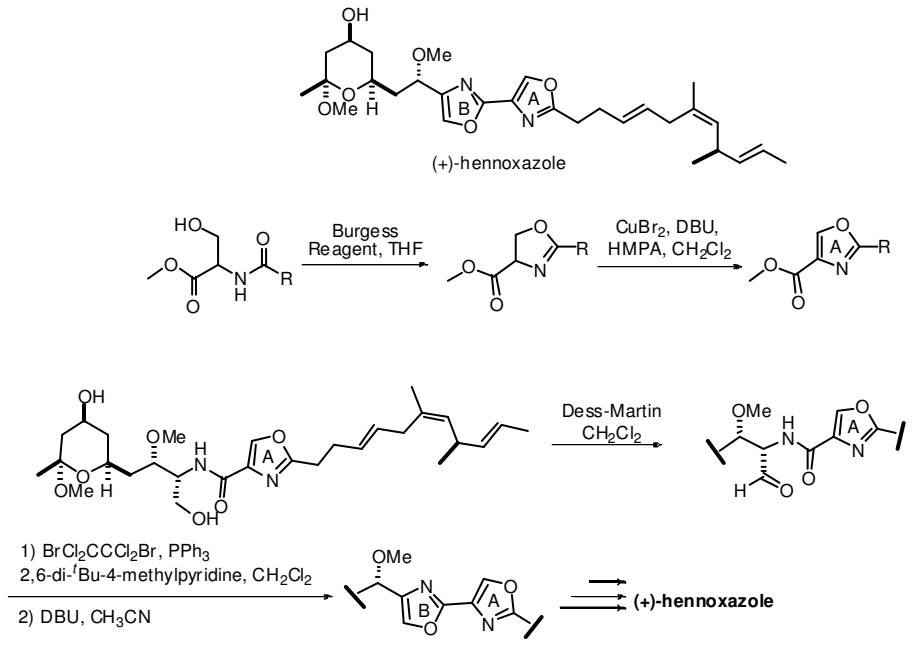


Figure 43: The structures of the bisoxazoles (-)-hennoxazole and muscaride A

The inclusion of azoles, particularly multiple linked azoles, into a total synthesis obviously provides a greater challenge than the synthesis of a single isolated oxazole. Many of the reagents and methods classically used for oxazole synthesis may be unsuitable for inclusion in a total synthesis, due to incompatibility with protecting groups and the need to conserve the stereochemistry in other areas of the molecules. New strategies to cope with these problems have been developed, of which a few are described below.

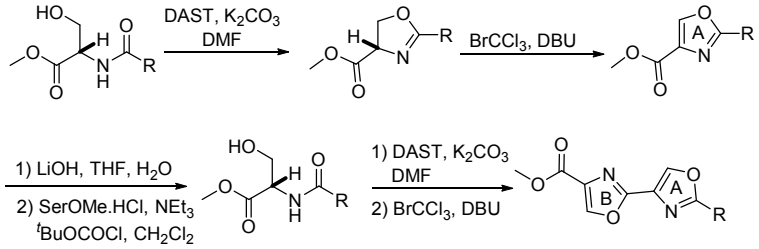
4.4.1 SYNTHESIS OF POLYOXAZOLE COMPOUNDS

Wipf's synthesis of (+)-hennoxazole (the enantiomer of the natural product, Scheme 24) involved an iterative bisoxazole synthesis.[45] The first serine residue was dehydrated using Burgess reagent to give the oxazoline, then oxidised using $CuBr_2$ and DBU to give the A oxazole unit. In contrast, the second serine-derived B oxazole unit was formed *via* oxidation to the aldehyde using Dess-Martin reagent, followed by cyclodehydration using 1,2-dibromotetrachloroethane, PPh_3 and an extremely hindered base, 2,6-di-*tert*-butyl-4-methylpyridine, which stopped at the intermediate 10-bromooxazoline. The AB bisoxazole unit was then obtained using a DBU mediated dehydrohalogenation reaction.[46]



Scheme 24: Wipf's synthesis of oxazoles A and B of (+)-hennoxazole[45]

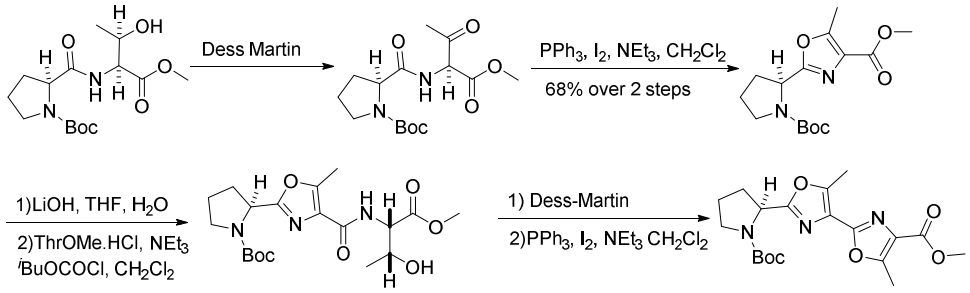
Alternative syntheses of the hennoxazoles have also used a convergent synthesis of the bisoxazole moiety, though the reagents differ.[47-49] Most simply, this was achieved by Williams *et al.* using a DAST mediated cyclodehydration and $BrCCl_3$/DBU oxidation to form the A oxazole unit, followed by a peptide coupling and repetition of these dehydration/oxidation condition to give the AB bisoxazole (Scheme 25).[47]



Scheme 25: Williams' synthesis of oxazoles A and B of (-)-hennoxazoles[47]

Wipf's synthesis of the threonine derived bisoxazole fragment of muscoride A was also obtained by an iterative sequence of oxidation and dehydration, using a Dess-Martin oxidation, followed by dehydration using $PPh_3/I_2/NEt_3$ (Scheme 26).[39, 46] This was found to be a superior strategy to tandem formation of the oxazoles, as the

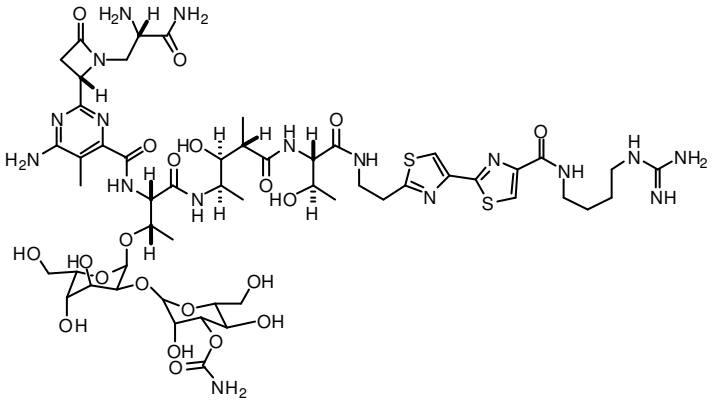
extremely polar tripeptide precursor (Pro-Thr-Thr) was difficult to handle in organic solvents.



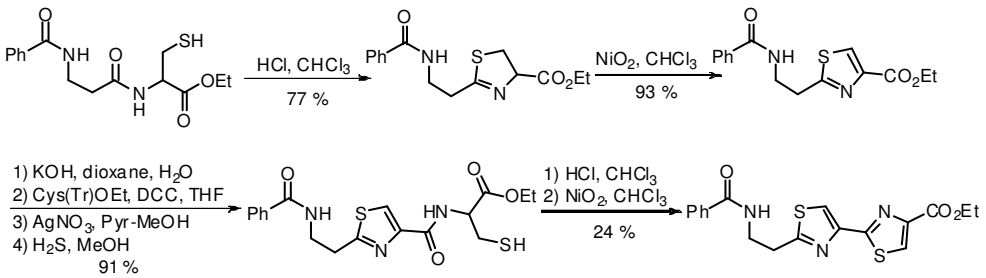
Scheme 26: Wipf's synthesis of the bisoxazole moiety in muscoride A

4.4.2 THIAZOLES IN NATURAL PRODUCTS

The inclusion of thioazoles in natural products requires further consideration. It is possible to synthesise them in a biomimetic fashion from cysteine, as performed by Hecht *et al.* in the synthesis of the bisthiazole unit of bleomycin B2 (Scheme 27).[50]



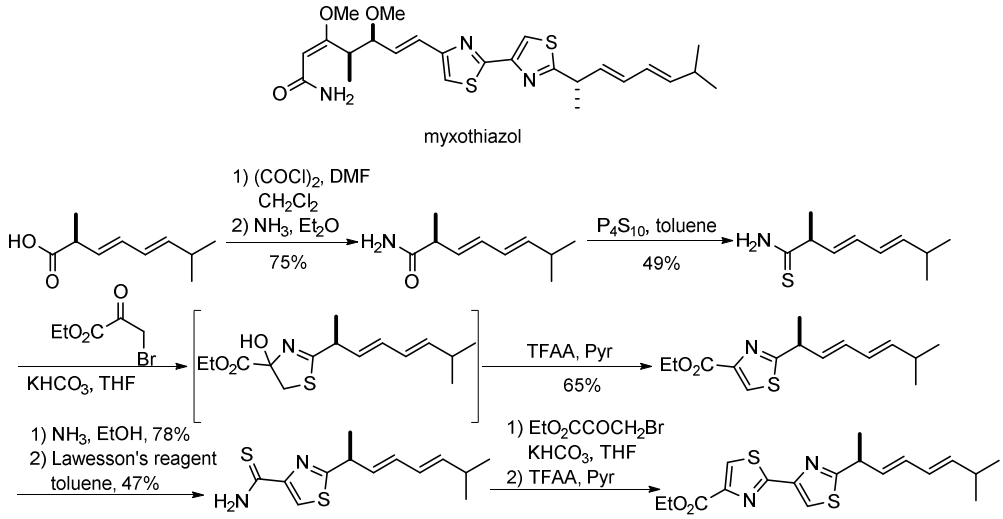
bleomycin B2



Scheme 27: The bisthiazole synthesis developed by Hecht *et al*[50]

The two thiazoles were synthesised in succession. Treatment of the cysteine residue with HCl gave the initial thiazoline, which was then oxidised using NiO_2 to give the first thiazole. Saponification of the ester was followed by a peptide coupling and deprotection of the cysteine thiol to give a tripeptide. This was dehydrated and oxidised as before, though in substantially poorer yields.

An alternative method was used by Pattenden *et al.* for the synthesis of the bisthiazole moiety of myxothiazol (Scheme 28).[51] This involved preparation of a thioamide, followed by a modified Hantzsch thiazole synthesis with ethyl bromopyruvate. This proceeded *via* the hydroxythiazoline, which was then dehydrated to give thiazole ester.[52] This sequence was then repeated to give the bisthiazole.



Scheme 28: The synthesis of the bisthiazole moiety of myxothiazol[51]

The Hantzsch thiazole synthesis has been used extensively in natural product synthesis,[52-55] but can be limited by the α-halo ketones used, meaning the thiazoles can only be built up sequentially.

4.4.3 TELOMESTATIN AND OTHER MACROCYLIC POLYAZOLES

The synthesis of natural products containing mixed threonine and serine-derived oxazoles and thiazoles adds a new level of complexity to the choice of reagents and route. Telomestatin is a complex macrocyclic polyazole derived from *Streptomyces anulatus* 3533-SV4 and has been shown to be a potent telomerase inhibitor.[56, 57] The total syntheses of this has been completed as both the (*R*)- and (*S*)- enantiomers by two groups, Doi *et al*.[58, 59] and Moody *et al.*[60] While both groups exploited the same disconnections, giving two trisoxazole building blocks, the method by which the oxazoles were synthesised were markedly different (Figure 44).

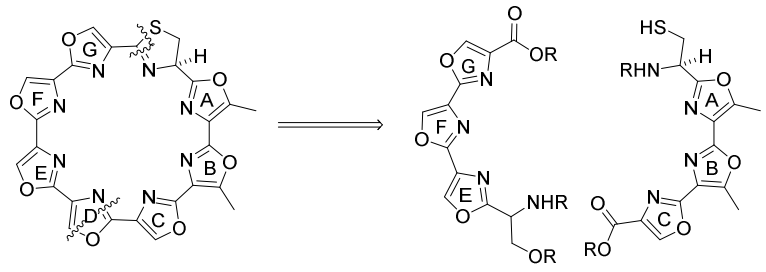
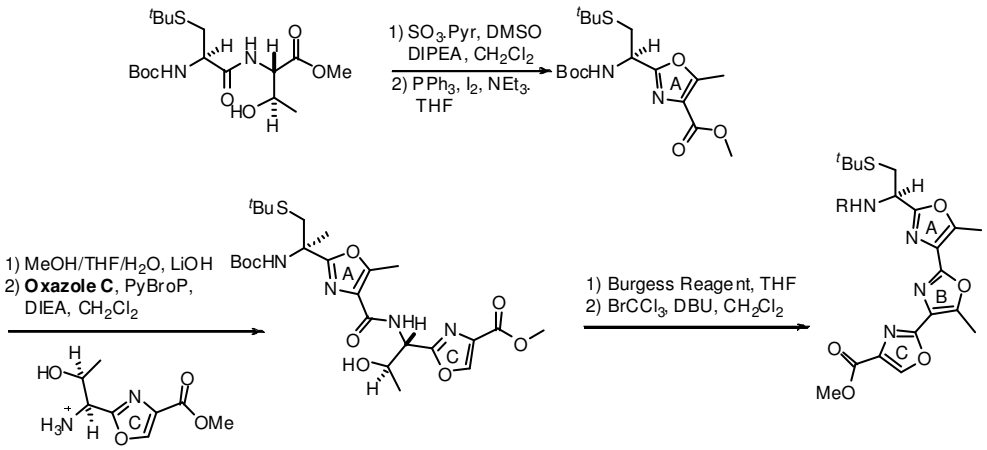


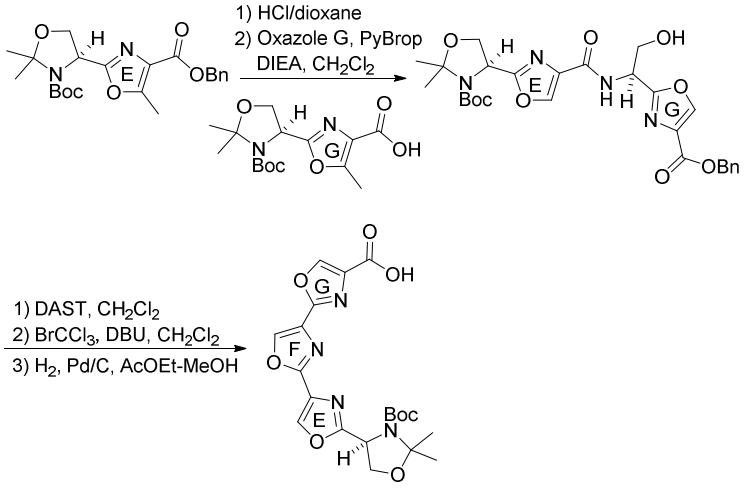
Figure 44: (*R*)-Telomestatin, and the common disconnections by Doi *et al*. and Moody *et al*.

In the synthesis of the natural (*R*)- and unnatural (*S*)-enantiomers, Doi *et al*. used relatively simple reagents to build up the two building blocks, based on three common synthetic units (Scheme 29). Oxazole A was synthesised using a Parikh-Doering oxidation,[21] followed by a $PPh_3/I_2/NEt_3$ dehydration, while synthesis of oxazole B used a Burgess cyclodehydration, followed by $BrCCl_3$/DBU mediated oxidation to give the 2 trisoxazoles.



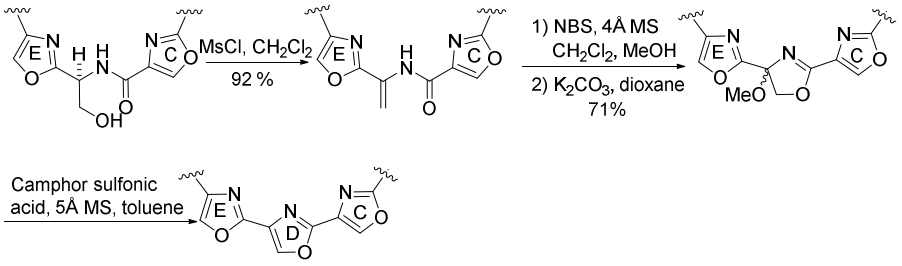
Scheme 29: Synthesis of the ABC trisoxazole by Doi *et al*

The serine derived oxazoles, G, F, E and C were synthesised using cyclodehydration with DAST and $BrCCl_3$/DBU mediated oxidation (Scheme 30).



Scheme 30: Synthesis of the EFG trisoxazole by Doi *et al.*

The two trisoxazole units were joined by the peptide bond formation between oxazole G and the cysteine residue. Following a peptide coupling between the deprotected serine residue of oxazole E and the deblocked carboxylic acid of oxazole C, attempts to form the oxazole D using the methods previously employed did not work. Instead, the serine residue was dehydrated and treatment of this alkene with NBS in the presences of 4Å molecular sieves afforded the α-methoxy-β-peptide. Dehalogenative cyclisation provided the methoxy-oxazoline which was dehydrated by treatment with camphor sulfonic acid to give oxazole D (Scheme 31).



Scheme 31: Formation of oxazole D

Finally, the sulfur was deblocked and the thiazoline formed by treatment with $PPh_3(O)$/TFAA/anisole to give (*R*)-telomestatin.

The synthesis by Moody *et al.* utilised a rhodium catalysed oxazole formation to build up the two trisoxazole units.[60] In this, the oxazoles were built up sequentially using reactive rhodium carbene intermediates in reactions with either a nitrile or a carboxamide (Figure 45). This synthesis showcases the robustness and utility of these transition metal reactions already seen in the total synthesis of a number of other oxazole conataining natural products,[27, 61-63] though the overall yields still do not approach those seen in more conventional approaches.

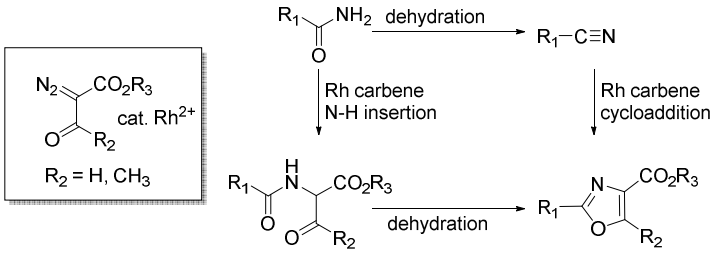


Figure 45: Routes to the rhodium catalysed synthesis of substituted oxazoles (adapted from Moody *et al.*[60])

We were particularly interested in the synthesis of two structurally related macrocycles, YM-216391 synthesised by Pattenden *et al.*,[32, 64] and IB-01211 by Albericio *et al.*,[65] as these have a number of structural similarities to azolemycin. Specifically, they contain a mix of oxazoles, amino-acid residues (YM-216391, Gly-Val-D-*allo*-Ile; IB-01211, Val-D-*allo*-Ile) and a thiazole flanked by oxazoles (**Figure 46**).

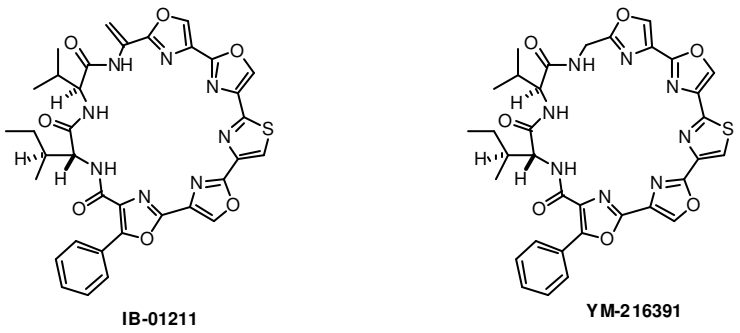
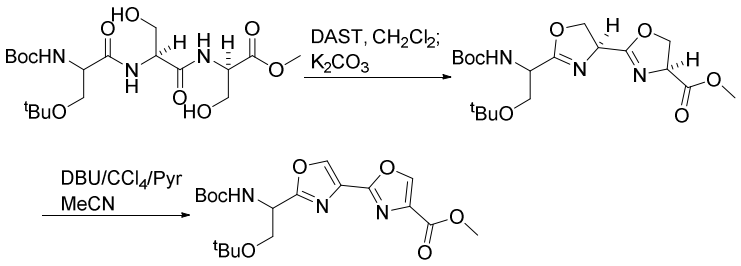


Figure 46: The structurally related IB-01211 and YM-216391

Both syntheses used a DAST cyclodehydration followed by an oxidation to form the four oxazoles, though Albericio *et al*. reported significant formation of the elimination product rather than the desired oxazoline when the β-phenyl-serine residue was treated with DAST. Interestingly, they also reported what they believe to be the first one pot formation of two concatenated oxazoles (Scheme 32) by cyclodehydration and oxidation. The oxidation of the concatenated oxazolines proved significantly more difficult than for the sequentially formed oxazolines, requiring the use of CCl_4/DBU in MeCN/pyridine to obtain the bisoxazole in 57% over 2 steps.



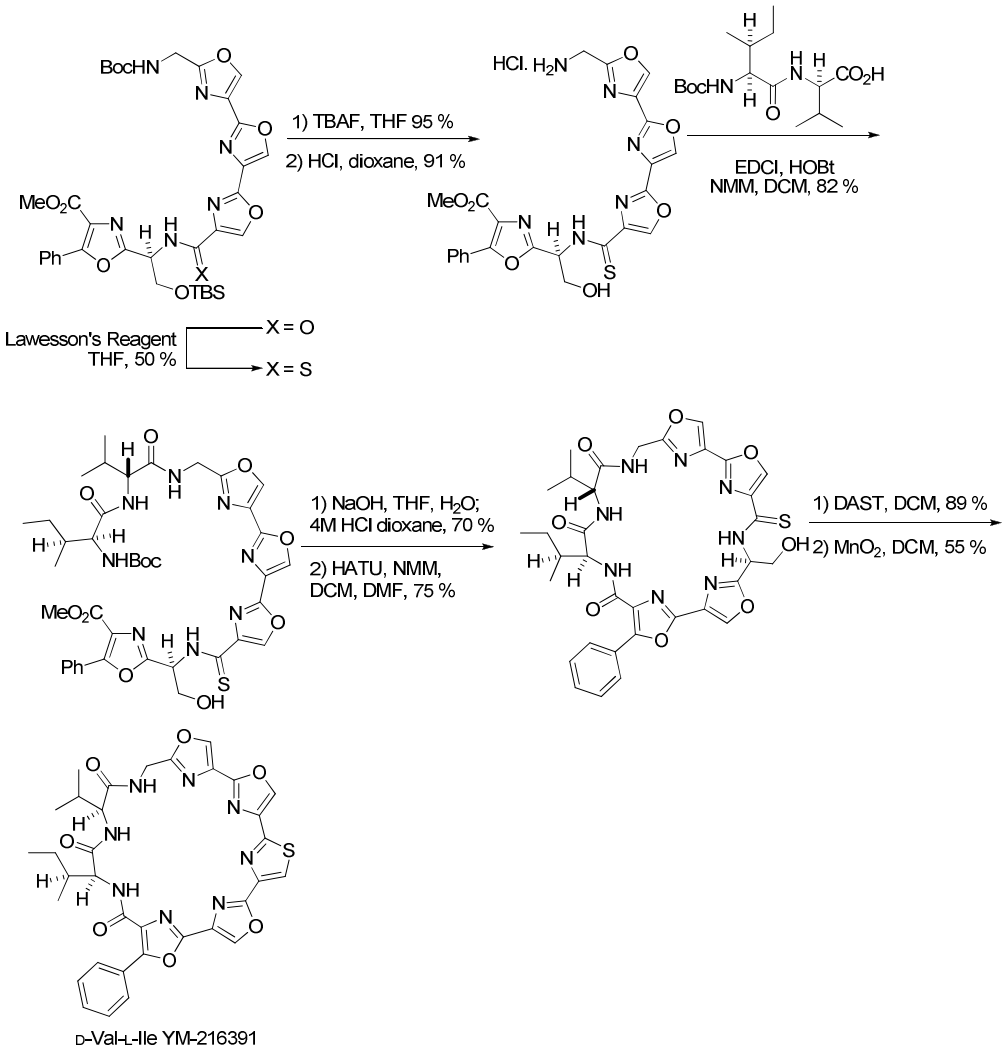
Scheme 32: The one-pot synthesis of the concatenated oxazoles by Albericio *et al*.

The syntheses of YM-216391 and IB-0211 differed significantly in the formation of the thiazole. Albericio *et al.* used a Hantzsch thiazole synthesis to effect the final macrocyclisation. Deprotection of the *C*-terminal bromo ketone, formation of the hydroxythiazoline and dehydration to the thiazole were performed in a one-pot process to give the final macrocycle in 11 % yield. Conversely, while Pattenden *et al.* also performed the thiazole formation last, they did not use it to close the macrocycle. Instead, a protected serine residue was carried through the synthesis,

before treatment with Lawesson's reagent to give the equivalent thioamide. Deprotection of the serine oxygen was followed by two peptide couplings to form the macrocycle before cyclodehydration gave the thiazoline. This heterocycle was oxidised with MnO_2 to give the thiazole and complete the synthesis (Scheme 33). In this case, late stage thiazole formation gave the additional conformational flexibility required for the formation of the macrocycle.

Scheme 33: Formation of the thiazole in a diastereomer of YM-216391 by Pattenden *et al*

An interesting sidenote to the synthesis of YM-216391 is the apparent discrepancy for the stereochemical assignment of the isoleucine. Sohda *et al.* assigned this as D-



allo isoleucine, though it is drawn as D-isoleucine in the article.[66] They were unable to differentiate between D- and D-*allo*-isoleucine by Marfey's analysis, but the

absolute configuration was assigned as D-*allo*-isoleucine by chiral HPLC. Pattenden *et al.* do not explicitly state their stereochemical assignment for natural (+)-YM-216391, but they originally synthesised it as the D-Val-L-Ile streeoisomer (-)-YM-216391, which gave an equal and opposite optical rotation to the natural product ((+)-YM-216391, $[\alpha]_D^{25}$ +48 (c = 0.1, CH_3CN); synthesised (-)-YM-216391, $[\alpha]_D^{20}$ -56 (c = 0.5, $CHCl_3$)). They therefore concluded that the compounds had the same relative stereochemistry, but exactly opposite absolute stereochemistry, implying the isoleucine is present in the natural product as the D-isoleucine rather than D-*allo*-isoleucine stereoisomer. In fact, a recent paper describing the biosynthesis of natural (+)-YM-216391 has again confirmed the stereochemistry as D-*allo*-isoleucine.[67] This further validates the utility of our method for differentiating between the relative diastereomers of isoleucine, especially in cases such as the synthesis of YM-216391, where the introduction of isoleucine occurs at a relatively late stage. In this case, it would be possible to produce the product as a mix of L- and D-*allo* isoleucylpeptides, allowing ready confirmation of the relative stereochemistry.

4.5 Approaches to the Total Synthesis of Azolemycin A

Having considered these previously synthesised natural products, especially the mixed polyazoles, we made the preliminary disconnections for the synthesis of azolemycin A. We immediately rejected a linear synthesis, as it would almost certainly be extremely low yielding, and instead focussed on a convergent synthesis, with late stage formation of the central thiazole, *cf.* the synthesis of YM-216391.[32] We therefore disconnected to give 4 distinct units: the isopropyl oxime **4.08**, the left hand mixed bisoxazole fragment **4.09**, the right-hand methyl oxazole **4.10** and the isoleucine methyl ester **3.14** (Figure 47).

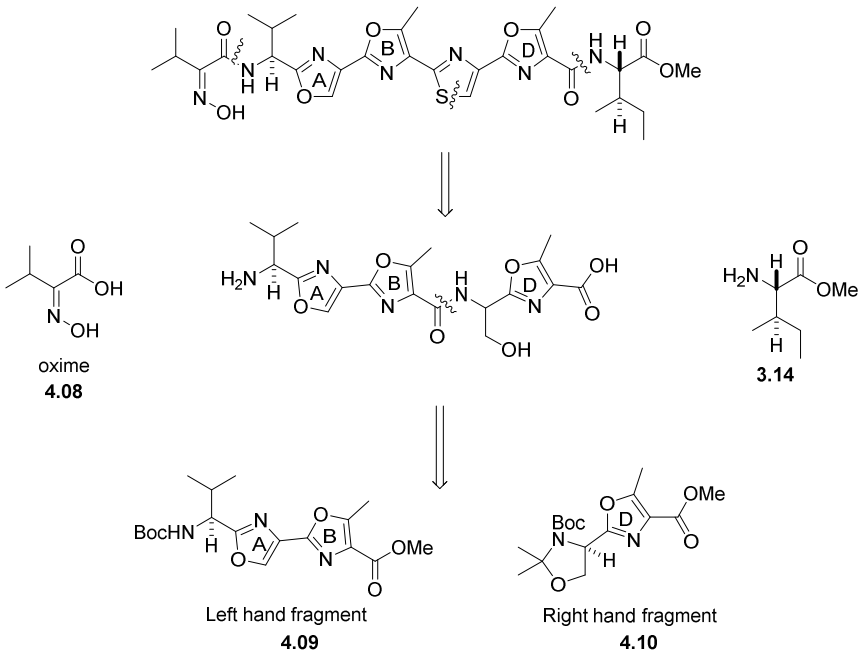
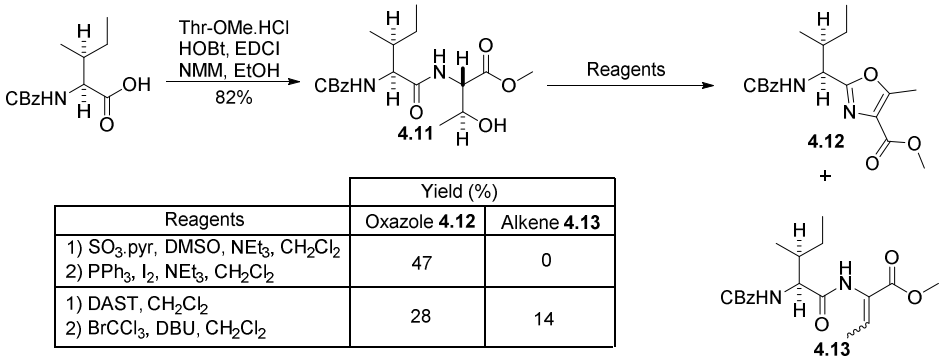


Figure 47: Preliminary disconnections for the synthesis of azolemycin A

4.5.1 Synthesis of Model Oxazoles

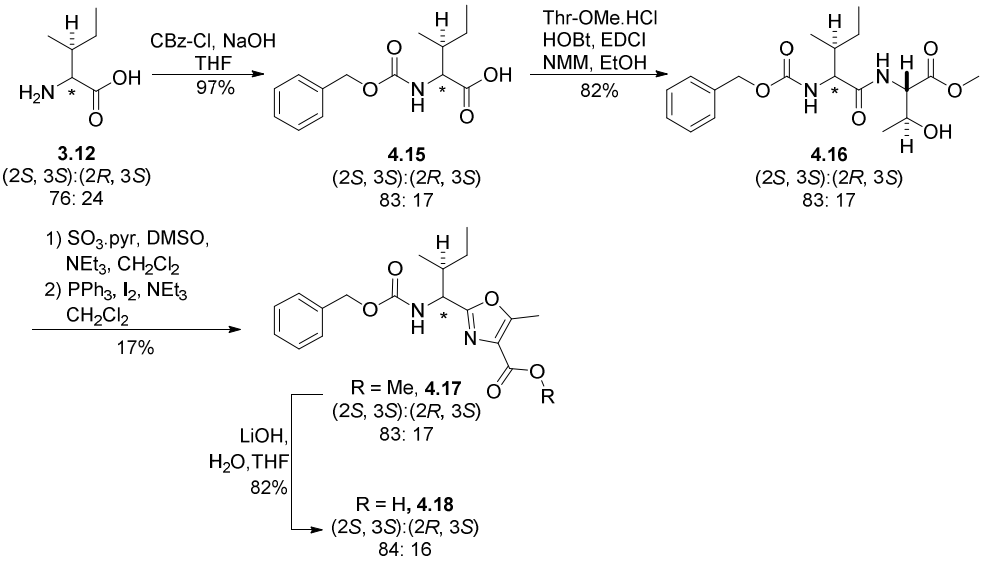
Our main concern at this early stage was preserving the stereochemistry of the *N*-terminal valine during the formation of oxazoles A and B. We therefore synthesised carboxylbenzoyl-L-isoleucyl-threonine-methyl ester **4.12** to assess the level, if any, of epimerisation occuring during the two oxazole syntheses (**Scheme 34**). The dipeptide was converted to the oxazole by the two different methods we hoped to use in the synthesis of azolemycin A. The first was the DAST mediated cyclodehydration, followed by a $BrCCl_3$/DBU oxidation. This gave the oxazole with no apparent epimerisation of the isoleucine α–stereocentre by ^1H NMR spectroscopy, though a significant amount of the elimination product **4.13** was also obtained in a ratio of around 2:1 (oxazole **4.12**: alkene **4.13**).



Reagents	Yield (%) Oxazole **4.12**	Alkene **4.13**
1) SO_3.pyr, DMSO, NEt_3, CH_2Cl_2 2) PPh_3, I_2, NEt_3, CH_2Cl_2	47	0
1) DAST, CH_2Cl_2 2) $BrCCl_3$, DBU, CH_2Cl_2	28	14

Scheme 34: The preparation of the model oxazole, **4.12**, as a single diastereomer

As we did not want to risk the formation of this elimination by-product during the synthesis of oxazole B, we also synthesised oxazole **4.12** using a different method. By first oxidising the threonine residue to a ketone, then performing a cyclodehydration, we hope to avoid this side reaction. Though there are a range of oxidation reagents available for this, we chose to follow the method set out by Doi *et al.*,[58] using a Parikh-Doering oxidation to give the β-ketoester. Whilst being mild and compatible with a range of functional groups, the Parikh-Doering oxidation has the advantage over the related Swern oxidation of being able to tolerate a wider range of temperatures, and reduced risk of by-products.[68] The cyclodehydration was performed according to the method developed by Wipf,[46] which provides a milder route to oxazoles compared to the traditional Robinson-Gabriel conditions.[26] This again produced oxazole **4.12** with no apparent epimerisation at the isoleucine stereocentre, and, as expected, in better yield than the DAST route. For comparison, the oxazole was also synthesised as a mix of diastereomers **4.17** from the previously epimerised isoleucine **3.12** (Scheme 35).



Scheme 35: Synthetic route to the model oxazole **4.17** as a mix of L-isoleucine and D-allo-isoleucine diastereomers

As noted previously, the ^{1}H signal for the isoleucine α-C<u>H</u> was sufficiently different for us to confirm that neither method of oxazole synthesis appeared to markedly affect the stereo integrity of the *N*-terminal amino-acid (Figure 48).

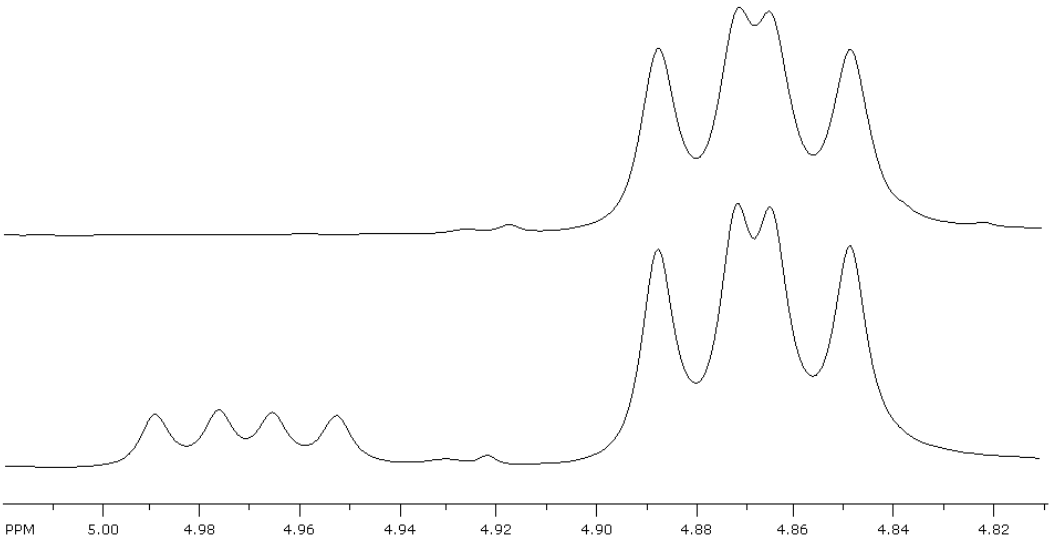
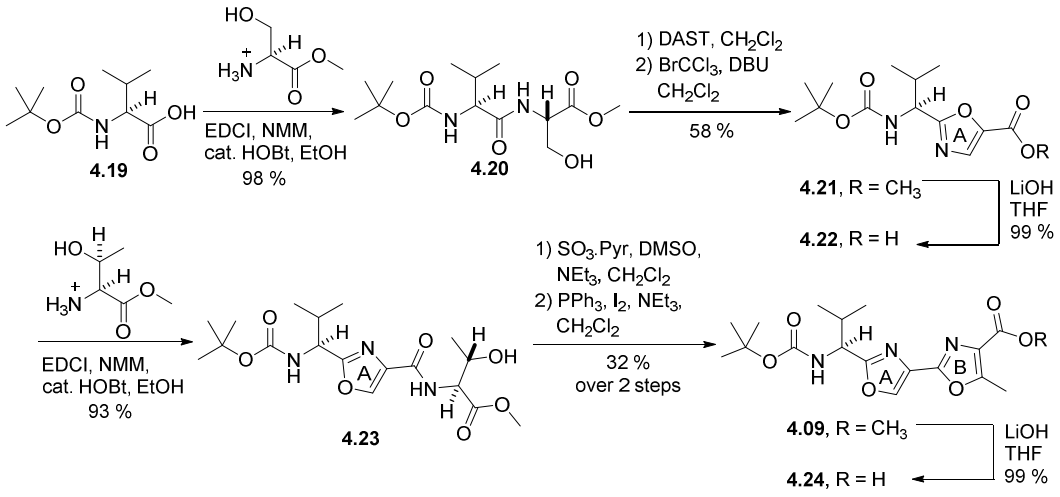


Figure 48: The dd peaks associated with the amide α-C<u>H</u> signal, showing the signal from L-isoleucine at around 4.80 ppm and the signal from D-*allo*-isoleucine at around 4.91 ppm from the reaction with the single diastereomer **4.12** (bottom spectrum) and the reaction with the mix of two diastereomers **4.17** (top spectrum)

Finally, to test the hydrolysis conditions needed to unblock the methyl ester, both the single diastereomer **4.12** and the mix **4.17** were hydrolysed to the corresponding acids **4.14** and **4.18** using lithium hydroxide. Again, no apparent epimerisation was observed by ^{1}H NMR spectroscopy.

4.5.2 Synthesis of the AB Bisoxazole Fragment

The preparation of model oxazoles **4.12** and **4.17** gave us confidence that we could bring the *N*-terminal valine though the synthesis (including peptide couplings, oxazole synthesis and hydrolysis) without risking significant epimerisation of the stereocentre. The initial dipeptide was formed by an HOBt catalysed peptide coupling in consistently high yields. Initially, the cyclodehydration to the intermediate oxazoline was performed using $CCl_4/PPh_3/NEt_3$ before oxidation with $BrCCl_3$/DBU to give the oxazole (Scheme 36). However, the stoichiometric quantities of triphenylphosphine oxide produced complicated the work-up, leading to disappointing yields. Switching to a DAST mediated cyclodehydration did not significantly increased the yield, but gave a more straightforward work-up so the reaction was easier to perform on larger scales.



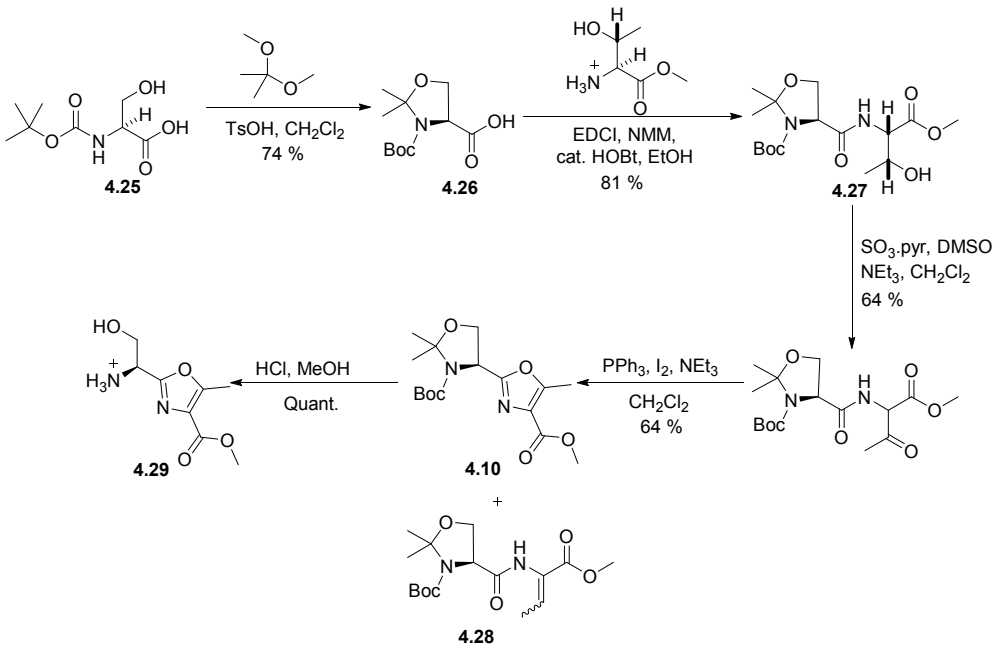
Scheme 36: The route to AB bisoxazole unit

Saponification of oxazole **4.21** and a further amide coupling gave threonine tripeptide **4.23** in excellent yields. Formation of the second oxazole proved more difficult than the first. The residue was first oxidised using the Parikh-Doering conditions before cyclodehydration with PPh_3/I_2. The yields were, at best, moderate and removal of the triphenylphosphine byproducts again proved an issue. However, attempts to use DAST to perform the initial dehydration gave a roughly 1:1 mixture of the elimination product and desired oxazoline. These had to be separated by column chromatography, as the alkene was extremely difficult to separate from the final oxazole if carried through to future steps, and the final oxazole yields were too low for this to be a viable alternative. Despite these issues, sufficient quantities of the AB bisoxazole unit **4.09** were synthesised to continue the synthesis.

4.5.3 Synthesis of the D Oxazole Dipeptide

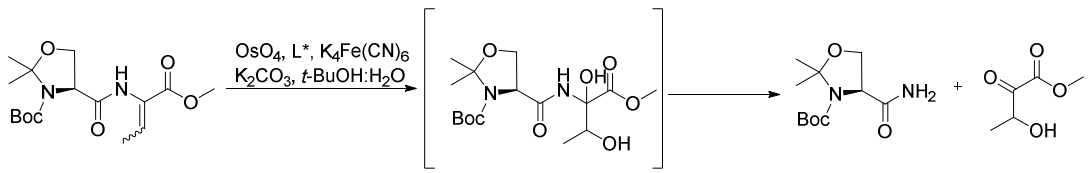
The *N*, *O*-protected serine derivative **4.26** underwent a peptide coupling to the threonine dipeptide **4.27** (Scheme 37) in excellent yields. As before, oxidation of the threonine residue using the Parikh-Doering conditions and cyclodehydration using $PPh_3/I_2/NEt_3$ gave the oxazole in moderate to low yields. It was not entirely obvious why the yields were so low, but there were a number of problems with both steps of the reaction.

It was difficult to track the progression of the oxidation, either by TLC or ^{1}H NMR, probably due to ready enolisation of the β-keto ester. Though the Parikh-Doering reaction is known to be more tolerant to increased reaction temperatures than the Swern oxidation, it was difficult to consistently maintain a low internal temperature when performed on a large scale, and this is known to have an effect on the yield of the reaction.[68] As noted previously, the cyclodehydration produced an equivalent of triphenylphosphine oxide, making the work-up and purification of the oxazole non-trivial, particularly on a multigram scale



Scheme 37: The synthesis of the D oxazole unit

When scaling up the oxidation we also found that removing excess starting material by silica chromatography was not straightforward, and in a number of batches some unreacted alcohol was carried through to the cyclodehydration. In these cases, the elimination product, **4.28**, was obtained and, as before, this was extremely difficult to separate from the desired oxazole. As the oxazole was not easy to synthesise in large batches, we turned to an unusual method to remove the alkene. A Sharpless asymmetric dihydroxylation was performed,[69, 70] which did not affect the oxazole, but gave what we assumed to be an extremely unstable diol (Scheme 38). As a simple aqueous work-up afforded only the clean oxazole, we assume this diol decomposed to give two water soluble by-products - an amide and a carbonyl ester.

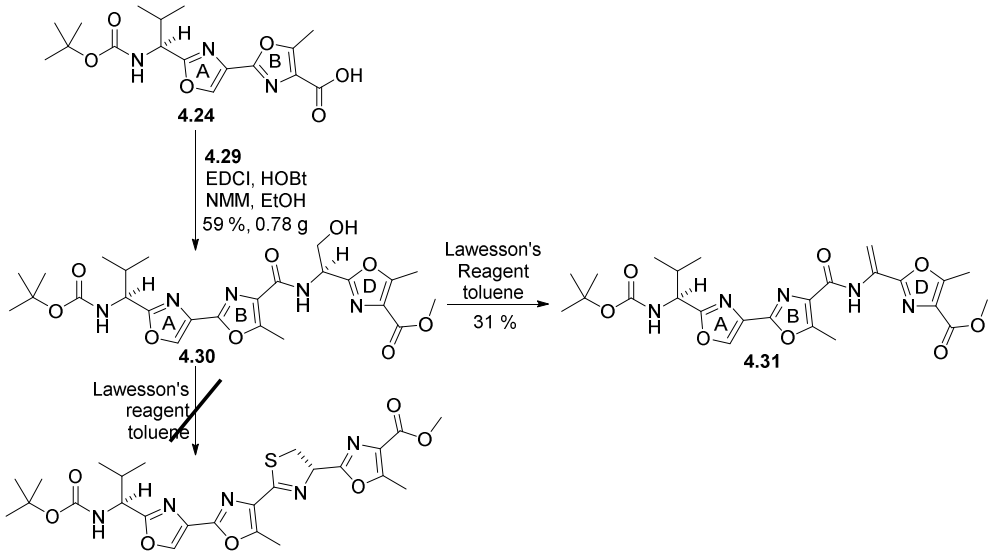


Scheme 38: The cleavage of the unwanted alkene **4.20** using a Sharpless asymmetric dihydroxylation (L* = $(DHQD)_2PHAL$)

4.5.4 SYNTHESIS OF THE PENTAPEPTIDE

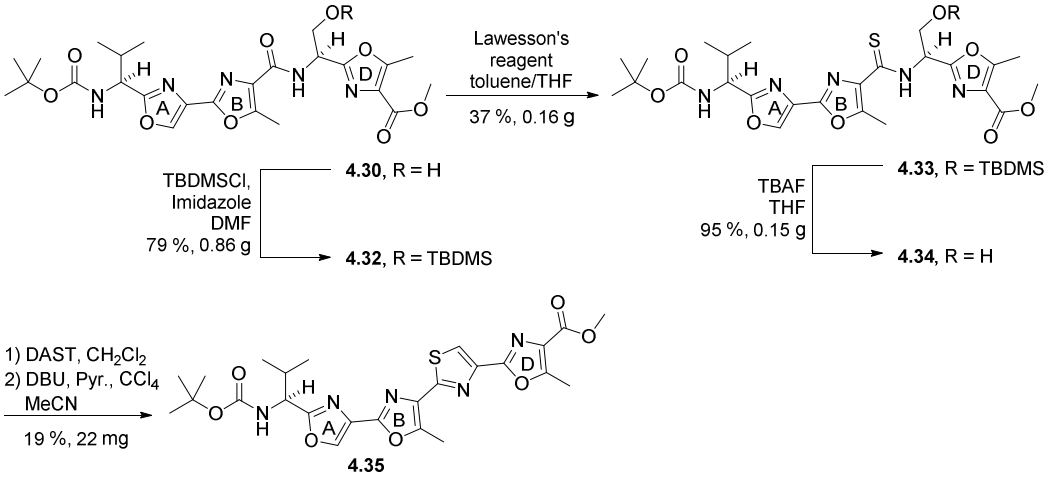
4.5.4.1 Lawesson's Reagent

The AB oxazole unit **4.09** was coupled to the deblocked serine residue of the D oxazole unit **4.29** in good yields using the HOBt/EDCI strategy previously employed (Scheme 39). Initially, we hoped to form the thiazoline in one step using Lawesson's reagent, according the procedure described by Zarantonello *et al.* in their preparation of althiomycin analogues.[71] However, the majority of the product obtained was the elimination product **4.31**, with no isolated thiazoline or thioamide.



Scheme 39: Attempted synthesis of the internal thiazoline

As this approach failed, we chose to undertake a slightly longer synthetic route, with a much lower risk of elimination. The serine alcohol was protected, and the thioamide formed using Lawesson's reagent. As suggested by the lack of thioamide formation in our previous attempt (Scheme 40), this was a relatively slow process, requiring around 40 hours reaction time at reflux in a combination of toluene and THF. Despite this, the thioamide was obtained in relatively high yields following purification by silica chromatography.



Scheme 40: Revised route to thiazole-containing pentapeptide

Conveniently, the ^{1}H NMR spectrum of the thioamide **4.33** was significantly different from the carboxamide precursor **4.32**. The chemical shift for the α-C14H adjacent to the thioamide group was around 0.5 ppm downfield, and the associated α-N13H nearly 2 ppm downfield compared to the equivalent carboxamide starting material (Figure 49). This allowed for rapid assessment of conversion from the crude ^{1}H NMR spectrum.

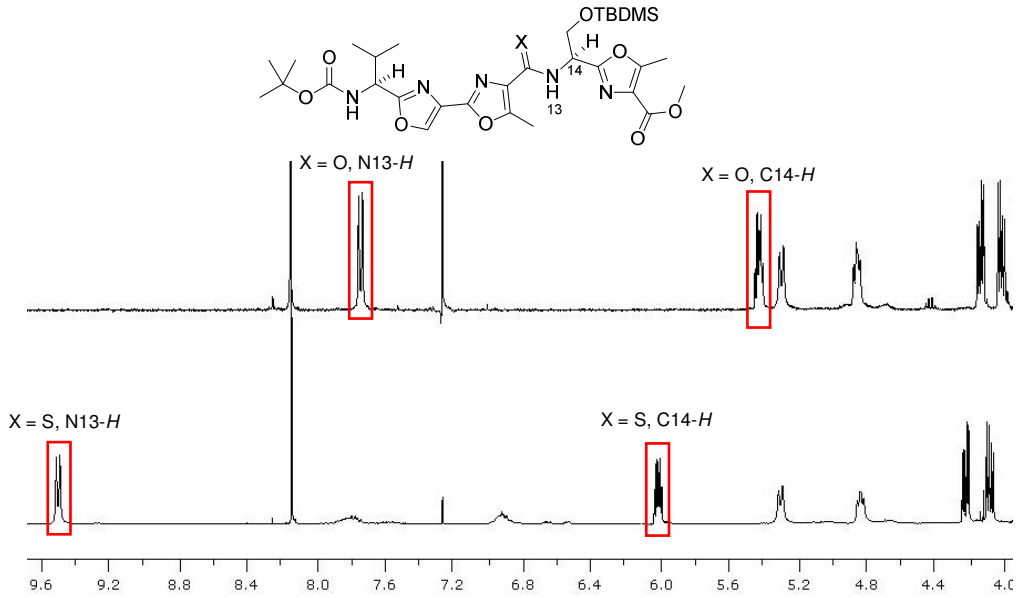


Figure 49: The ^{1}H NMR spectra for the thioamide **4.33** (bottom spectrum), highlighting the CH and NH shifts, and the equivalent carboxamide starting material **4.32** (top spectrum)

4.5.4.2 Formation of the Thiazole

Following deprotection of the alcohol, the thiazoline was obtained using the same DAST cyclodehydration strategy as for the serine derived oxazolines (Scheme 40). However, the previous oxidation conditions ($BrCCl_3$/DBU) did not appear to be sufficient, with incomplete thiazole formation observed even in the case of large excesses of reagent. Instead, a modified oxidation procedure using CCl_4/pyridine/DBU was utilised, according the method of Jung *et al.*[72] This route allowed us to complete the synthesis of the pentapeptide polyazole core **4.35**, but initial efforts yielded only 22 mg, insufficient to reliably complete the total synthesis.

4.5.5 REVISED SYNTHESIS OF THE PENTAPEPTIDE CORE

Our initial approach to the pentapeptide core **4.35** (Figure 47), though successful, suffered from low yielding reactions early in the synthesis, particularly in the production of the AB oxazole unit **4.09**. While re-examining the synthetic route, we noticed symmetry within the molecule that we had missed during our preliminary disconnections. As the thiazole was derived from a serine residue, the tetraazole core could be divided into two repeating serine-threonine dimers, corresponding to the previously synthesised oxazole D **4.10** (Figure 50). We therefore revised our disconnections using oxazole D as a common precursor. An additional benefit to this synthetic route was the late addition of the *N*-terminal valine, reducing even further the possibility of epimerisation of the stereocentre.

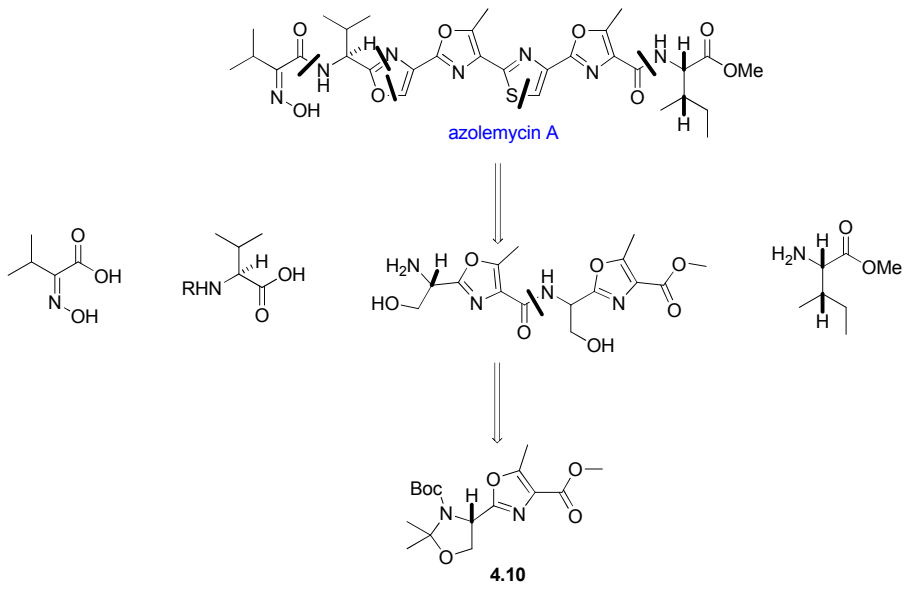
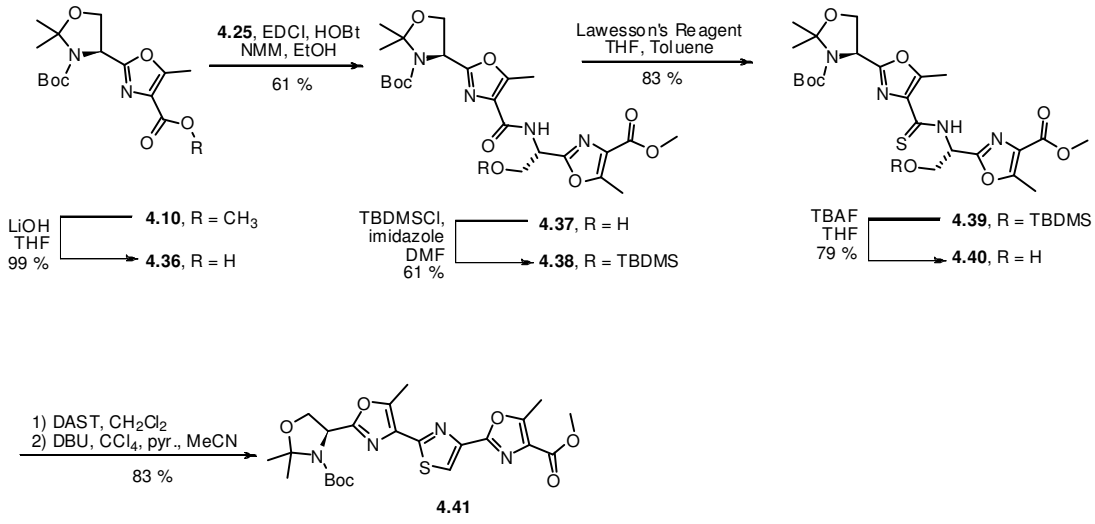


Figure 50: Revised disconnections for the pentapeptide

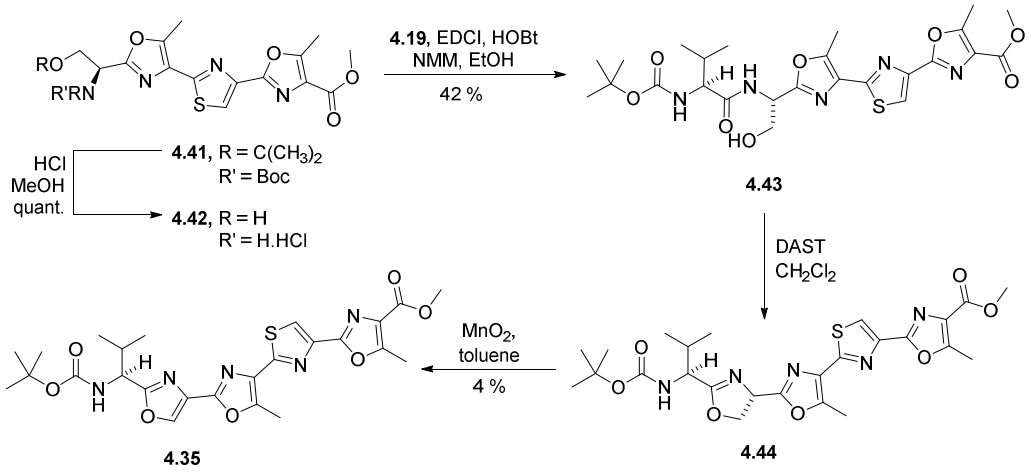
4.5.5.1 Synthesis of the BCD unit

Following an EDCI/HOBt mediated peptide coupling between two differently deprotected oxazole units **4.25** and **4.36**, thiazole **4.41** was installed using the same chemistry as before (Scheme 40) to give the protected BCD trisazole unit (Scheme 41).



Scheme 41: Synthesis of the BCD azole unit

Deblocking of the *N*-terminal serine residue and a further peptide coupling installed the *N*-terminal valine residue **4.19** (Scheme 42). This was readily converted to the corresponding oxazoline using DAST, and attempts were then made to convert the crude mixture to the oxazole. However, neither of the previously employed oxidation strategies (DBU/$BrCCl_3$ or DBU/Pyr/CCl_4) resulted in any significant oxazole formation. Some conversion was noted with commercial activated MnO_2, but the yields were extremely low (<1 %) and irreproducible.



Scheme 42: Revised synthesis of the pentapeptide **4.35**

We expected the oxidation to be comparatively more difficult than for oxazolines with an electron withdrawing group at the 5-position, but considering the relative ease of the oxidation for the internal thiazole **4.41** (Scheme 41) we did not anticipate such difficulty. It appears that, while an electron withdrawing group at the 5-position, especially an ester, is important for the oxidation of oxazolines (where $CuBr_2$ is used, it is apparently essential), the reaction also benefits from conjugation with neighbouring azoles. In the case of the oxazoline **4.44**, there is neither significant conjugation nor a strong electron withdrawing group. Though this would be expected to disfavour oxazole formation, we were encouraged by the small quantity of oxazole produced by MnO_2 oxidation. Though oxidations of this type of unactivated oxazoline are not common in the literature, examples do exist which utilise either MnO_2 or NiO_2,[20] both of which are assumed to follow a similar

mechanism.[73-76] As the commercial activated MnO_2 gave very poor results, freshly activated MnO_2 was synthesised according to the method of Carpino *et al*.[77] Activated carbon is oxidised by potassium permanganate, and the resulting active MnO_2 is deposited on the remaining unoxidised carbon. Using this preparation, the product oxazole **4.35** was obtained, but the yield was still extremely low (4 %).

4.5.5.2 Manganese Dioxide

Whilst the second set of disconnections provided some significant advantages over our first synthetic attempt, namely providing a more convergent synthesis a common building block to build the central pentapeptide, it also suffered from extremely poor yields. This was primarily due to the poor conversion of the final oxazoline to form oxazole A. We were hopeful that by installing this oxazole unit earlier, and optimising the MnO_2 oxidation for this conversion, we could complete the synthesis of the pentapeptide in sufficiently high yields to progress the synthesis. We decided that the convenience of having a common building block **4.29** overruled the problems associated with the oxidation of the relatively unactivated *N*-terminal oxazoline. In order to mitigate the low yield, we decided to install this earlier in the synthesis, choosing to use oxazole **4.29** as a building block for an alternative synthesis of the AB oxazole unit **4.09** (Figure 51).

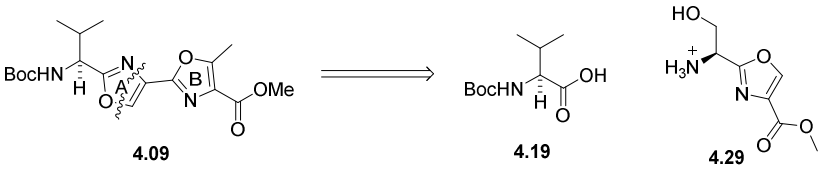


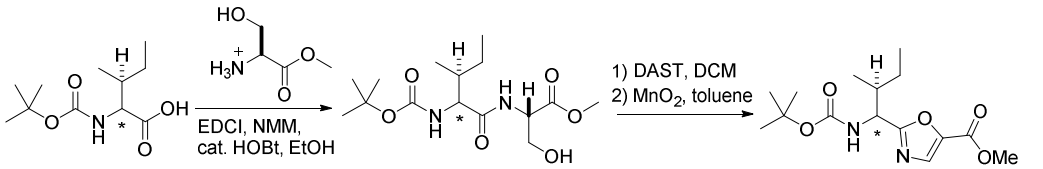
Figure 51: Revised disconnections for the AB bisoxazole

We had already found that the MnO_2 prepared according to Carpino's procedure gave better results that the commercially obtained reagent. Despite being a widely used oxidising agent, many procedures do not state the provenance or preparation method of MnO_2, and the method of preparation has been shown to have a significant effect on the activity.[78, 79] Common procedures involve either the

pyrolysis of a range manganese salts at high temperatures,[80, 81] or, more conveniently, by the precipitation of MnO_2 from the reaction of a solution of potassium permanganate and manganese sulfate in acidic or basic conditions.[82-84] These give preparations of varying activities, and it is reported that the activity can be increased by washing with dilute nitric acid.[81] In addition, MnO_2 can be conveniently deposited onto silica,[85] aluminium[86] or, as previously described, carbon.[77]

To our knowledge, there is no information regarding the risk, if any, of epimerisation of amino-acid stereocentres during MnO_2 oxidations. Though not very likely, the prolonged heating required for the transformation prompted us to perform the reaction first on a model isoleucine compound, to ensure the stereointegrity of the *N*-terminal valine could be maintained.

The Boc-protected dipeptide was synthesised as both the single isoleucine L-diastereomer **4.47** and a mix of L-isoleucine and D-*allo*-isoleucine diastereomers **4.48** (Scheme 43). The oxazoles were prepared by a DAST cyclodehydration, followed by heating with a large excess of MnO_2, prepared as the Carpino method.



4.45, (2*S*, 3*S*)
4.46, (2*S*, 3*S*: 2*R*, 3*S*), 73: 27

4.47, (2*S*, 3*S*); 60 %
4.48, (2*S*, 3*S*: 2*R*, 3*S*), 78: 22; 76 %

4.49, (2*S*, 3*S*); 2 %
4.50, (2*S*, 3*S*: 2*R*, 3*S*), 78: 22; 1 %

Scheme 43: Preparation of the model isoleucine containing oxazoles

Encouragingly, we could see no evidence of epimerisation of the isoleucine α-stereocentre by ^{1}H NMR (Figure 52) but the yields and conversion of the oxazole were still poor.

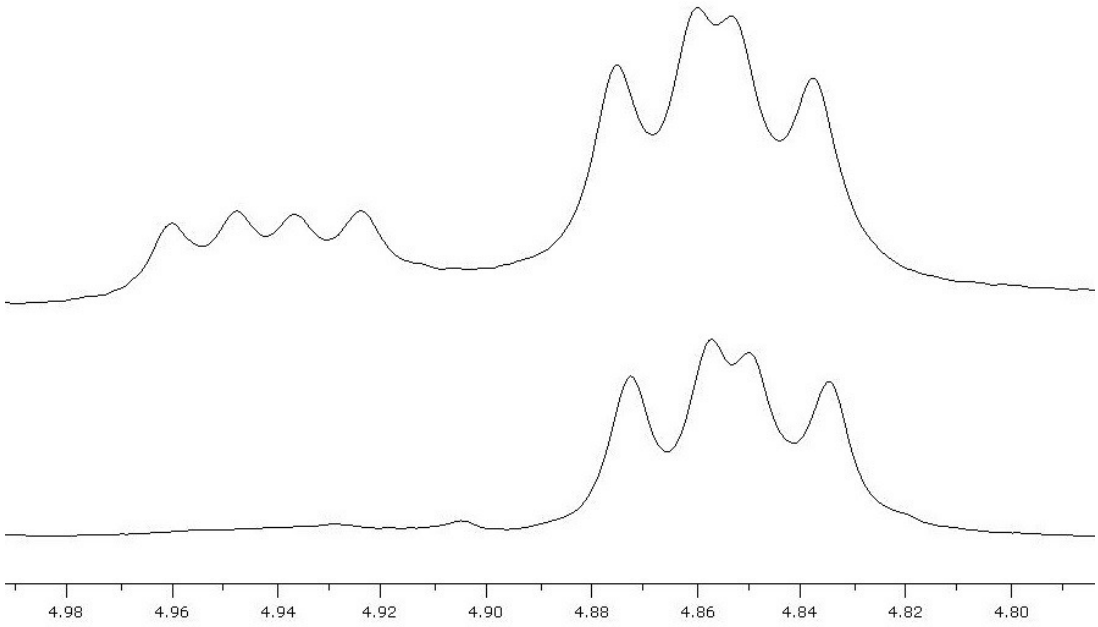


Figure 52: The dd peaks associated with the amide α-C<u>H</u> signal, showing the signal from L-isoleucine at around 4.86 ppm and the signal from D-*allo*-isoleucine at around 4.96 ppm from the reaction with the single diastereomer, **4.49** (bottom spectrum) and from the reaction with the mix of two diastereomers, **4.50** (top spectrum)

The conversion of the crude reaction was, at best, 50 % over reaction times of around 20 hours, and though the oxazoline and oxazole were easily separated by silica chromatography, the isolated yields were much lower than would be expected (1 - 2 %). ^{1}H NMR showed no obvious by-products being formed competitively, and it appeared that the low yield was partially caused by the product complexing with the MnO_2.

In order to improve both the yield and the conversion, we investigated a second method of producing activated MnO_2. Following a method proposed by Ball, Goodwin and Morton, an aqueous solution of potassium permanganate was reacted with manganese sulfate in neutral medium to give a precipitate of MnO_2.[84] This was then activated as required by azeotropic drying with toluene, as described by Goldman.[87] Unlike Carpino's method, this gave us a precipitate of pure MnO_2, which could be reduced after completion of the reaction to give water soluble manganese sulfate and manganese dithionate, allowing for an aqueous work-up. In

this case, the reduction of MnO_2 was performed by aqueous SO_2, produced *in situ* from a solution of H_2SO_4 and sodium bisulfite (Equation 1).

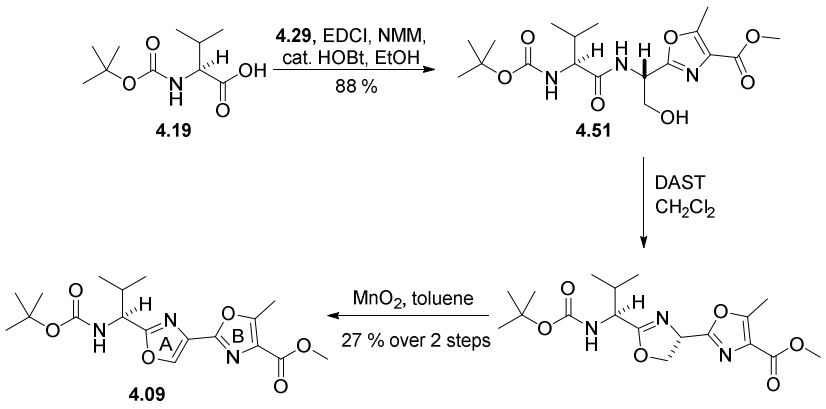
$$H_2SO_4 + Na_2SO_3 \rightarrow Na_2SO_4 + SO_2 + H_2O$$

$$3SO_2 + 2MnO_2 \rightarrow MnSO_4 + MnS_2O_6$$

Equation 1: The formation of sulphur dioxide and subsequent reaction with manganese dioxide[88]

4.5.5.3 Revised preparation of AB Oxazole Unit

With an improved preparation of MnO_2 in hand, we again attempted the synthesis of the AB oxazole unit **4.09**. Again, the peptide coupling to give the tripeptide **4.51** progressed smoothly, as did the DAST-mediated cyclodehydration to give the crude oxazoline (Scheme 44). The MnO_2 oxidation was performed using the crude unactivated MnO_2, prepared according to the method Ball, Goodwin and Morton (*vide supra*). This was calculated as ~50 weight % water by mass. Residual water was removed using Dean-Stark apparatus, before addition of the oxazoline as a solution in toluene, and the reaction was complete by ^{1}H NMR spectroscopy within 6 hours at 110 °C. Reduction of the MnO_2, aqueous work-up and silica chromatography gave the clean bisoxazole **4.09** in 27 % yield over 2 steps, a great improvement of the oxazole formation of the pentapeptide **4.35** (Scheme 42). It appears that the majority of product was lost in the aqueous work-up, so a more careful approach to the removal of the MnO_2, or use of more robust protecting groups, could make this an extremely useful approach to unactivated oxazoles.



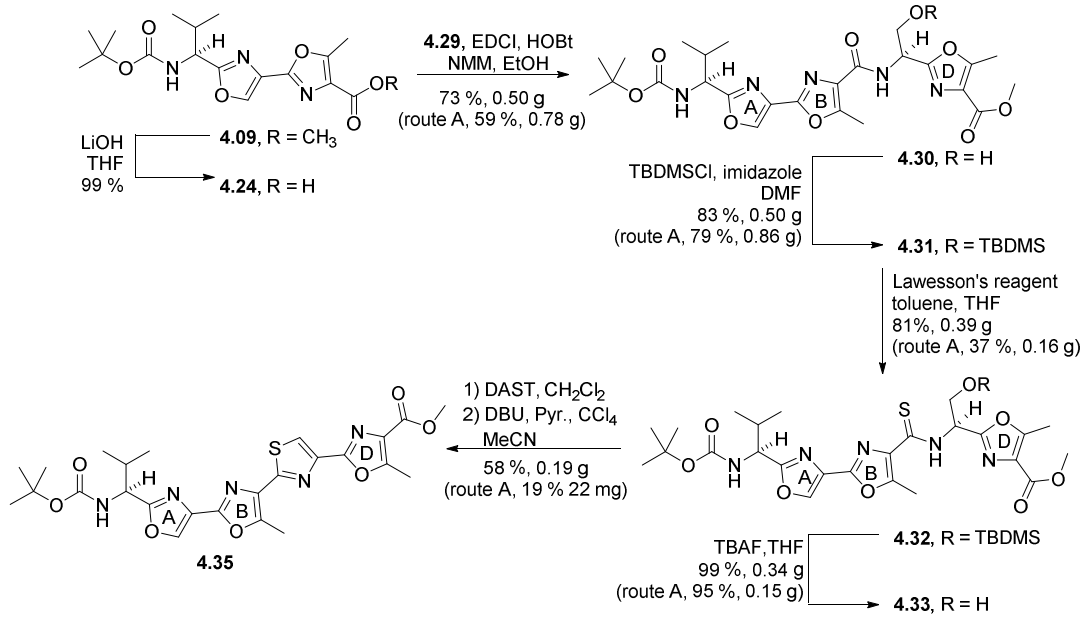
Scheme 44: The revised route to the AB bisoxazole unit

This route, though not ideal, provided the AB bisoxazole unit in sufficiently high quantities to complete the synthesis.

4.5.6 COMPLETION OF THE AZOLEMYCIN SYNTHESIS

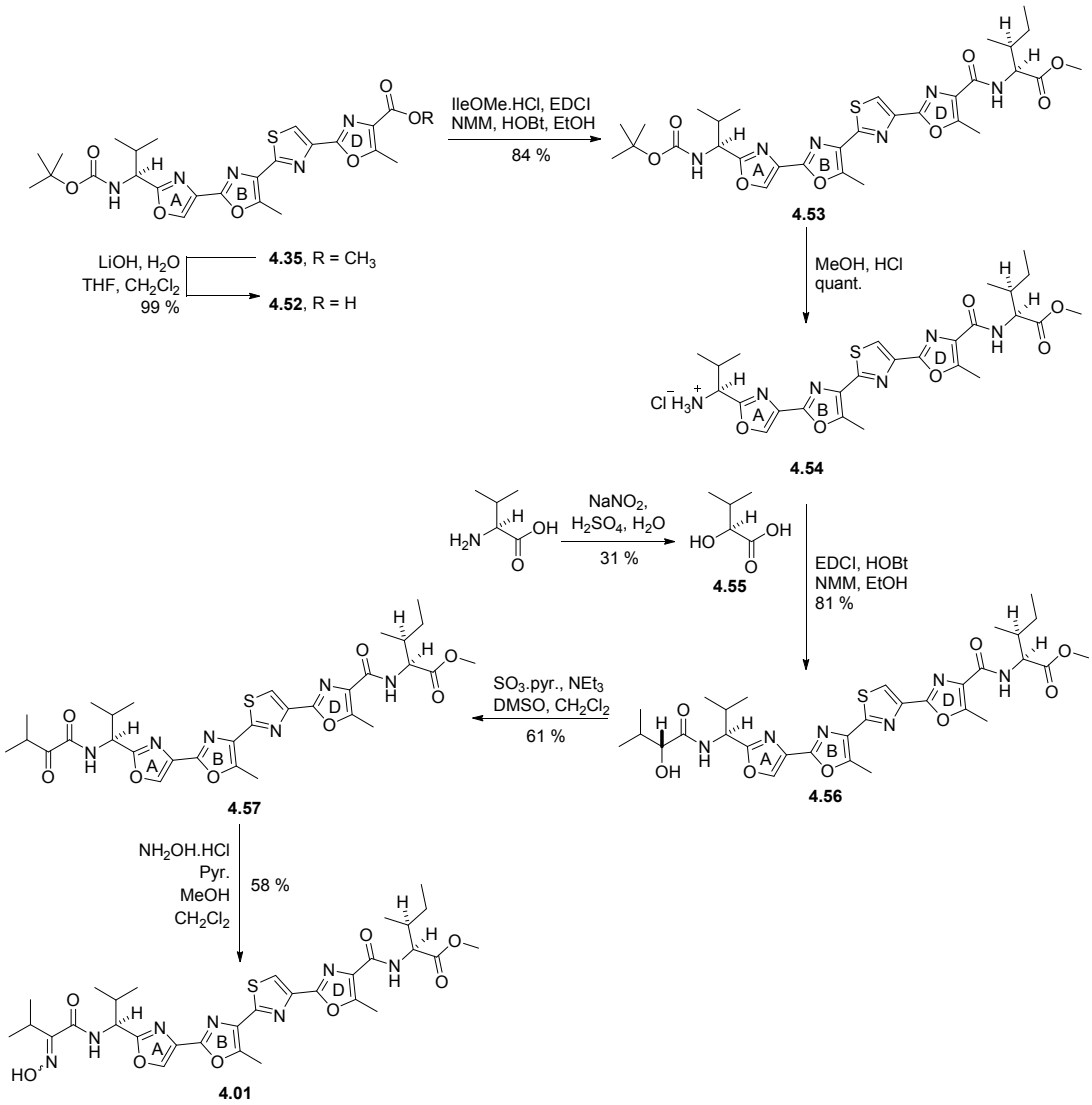
The synthesis of the bisoxazole AB fragment, **4.09**, using the revised method, although still relatively low yielding, was substantially easier that the original method. Ideally, we would have liked to optimise the synthesis, firstly by improving the synthesis of the oxazole D building block **4.10**, and secondly by improving the work-up for the MnO_2 oxidation. However, due to time constraints, we decided to continue the synthesis without optimisation.

The pentapeptide was successfully synthesised using the same route as before (Scheme 45). The overall yields were much improved compared to our first attempt, mianly to improvements in purifying the thioamide **4.32** and hence being able to perform the later reactions on a larger scale, giving the final polyazole in nearly tenfold greater yield than the first attempt.



Scheme 45: The third, successful, route to the pentapeptide **4.35**, showing the yields from the previous attempt (route A; Scheme 39, Scheme 40)

The saponification of the methyl ester was much less straightforward than for previous compounds, due to the extremely non-polar character of the polyazole and required long reaction times, extremely low dilution and a mix of solvents for dissolution and complete reaction (Scheme 46). Once formed however, the resulting acid **4.52** was readily coupled to the *C*-terminal isoleucine residue to give the hexapeptide **4.53**. L-Valine was converted to 2-hydroxyl-isovaleric acid **4.55** and coupled to the deblocked valine residue to give the alcohol precursor **4.56**, which was oxidised to the ketone **4.57** using Parikh-Doering conditions. Finally, the oxime was installed to provide azolemycin A **4.01** in 21 steps (16 steps, longest linear sequence), and an overall yield of 0.3 %.



Scheme 46: Route to the synthesis of azolemycin A

Comparison of the ^{1}H spectrum of the synthesised product with that of the extracted natural product showed extremely close correlation (Figure 53).

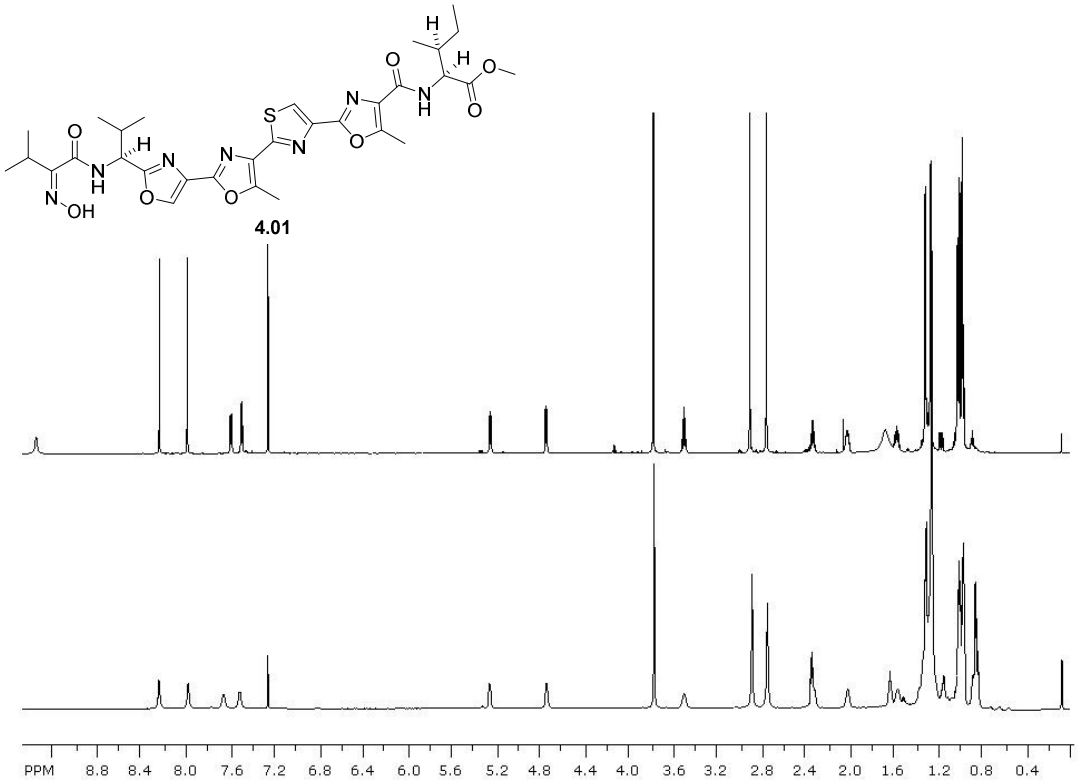


Figure 53: Top Spectrum: The ^{1}H NMR spectra for the synthetic azolemycin A, **4.01**, prepared as the *cis* isomer. **Bottom Spectrum:** The ^{1}H NMR spectra of the natural product azolemycin A from bacterial extract as the *cis* isomer

While the naturally extracted product was isolated separately as both the *cis* and *trans* geometric isomers, this was not possible for the synthesised product. In fact, the major product isolated was the *cis* oxime, with traces of the *trans*, based on the valine α-C<u>H</u> peaks on ^{1}H NMR spectra. However, as it is likely that the two isomers will interconvert in solution we were not concerned about this.

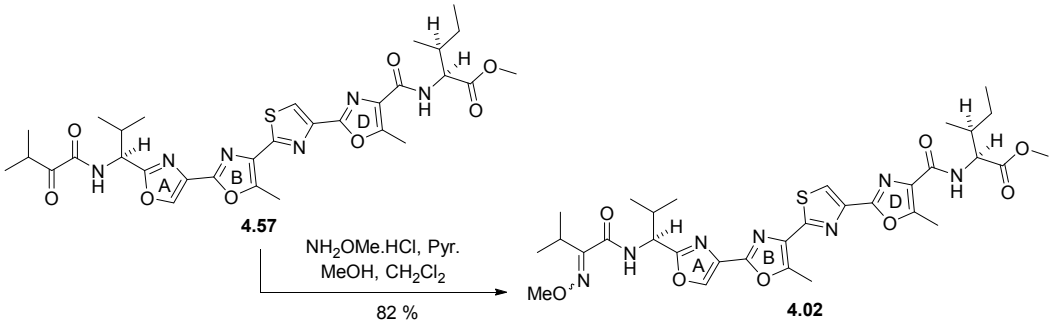
There had been insufficient natural product obtained by bacterial extract to obtain a ^{13}C NMR spectrum, and the carbon chemical shifts had been obtained by analysis of the HMBC and HMQC spectra. Comparison of the ^{13}C NMR spectrum obtained for the synthetised product with the carbon signals in near complete agreement, with the only discrepancy occurring in the assignment of the chemical shift for the δ-methyl group of isoleucine, given as 18.8 ppm for the bacterial extract but identified as 11.5 ppm for the synthesised product. The lower shift was confirmed as correct by HMQC, HMBC and by comparison with other isoleucine compounds.

4.5.7 BIOLOGICAL TESTING

At present, no biological activity has been identified for azolemycin A, as the limited quantities afforded by bacterial extraction restricted the possible assays required to identify this. However, our synthetic route presented us with sufficient quantities to allow a much greater range of assays to be run in future.

4.6 SYNTHESIS OF AZOLEMYCIN B

The methoxy analogue of azolemycin **4.02** was also synthesised using the same conditions as the hydroxyl product **4.01** (Scheme 47).



Scheme 47: Formation of the methoxy analogue of azolemycin A

This was obtained as primarily the *trans*-geometric oxime isomer, but in this case the interconversion between the oxime isomers was much faster than for the hydroxyoxime 4.01, with an NMR sample showing near complete conversion to the *cis*-isomer in around 5 days. Comparison of the ^{1}H NMR spectra of these two geometric isomers with the corresponding natural product extracts showed good correlation between the synthesized and natural products (Figure 54, Figure 55).

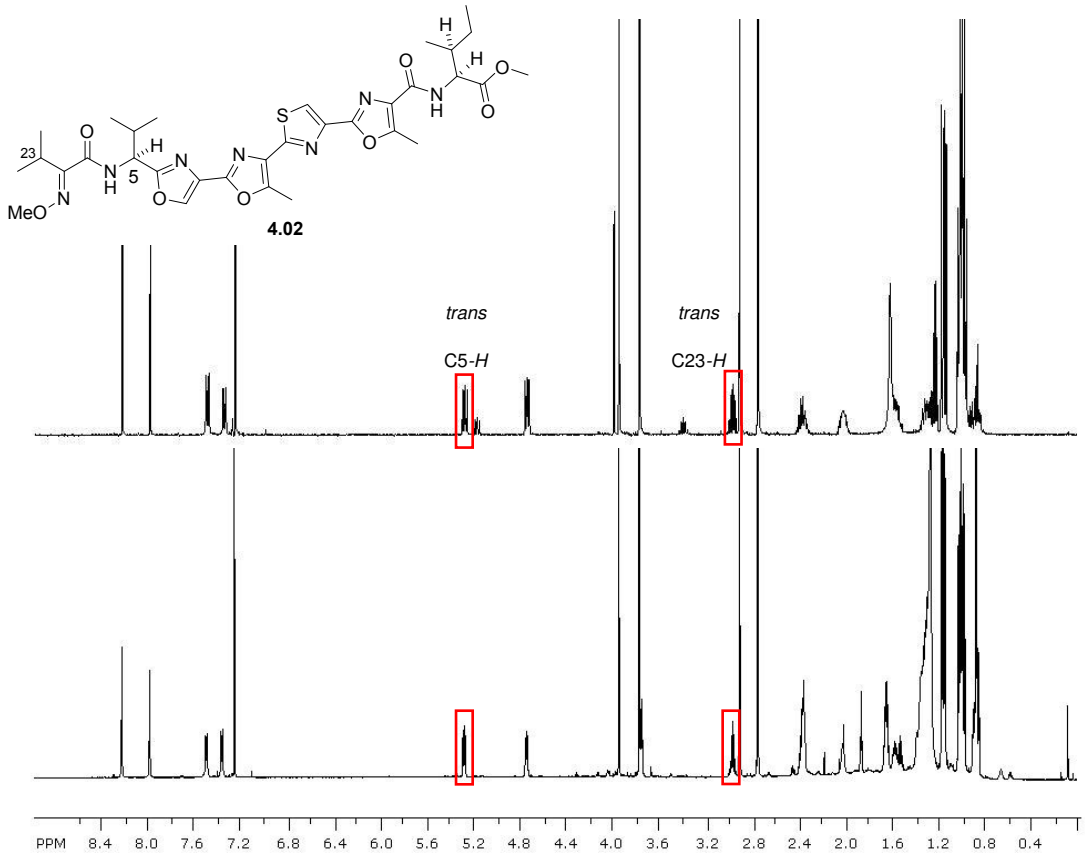


Figure 54: Top Spectrum: The ^{1}H NMR spectra showing the synthetic methoxyoxime product **4.02** as predominately the *trans* isomer (obtained on 400 MHz machine). **Bottom Spectrum**: The ^{1}H NMR spectra showing the natural product, azolemycin B, as the *trans* isomer (obtained on 700 MHz machine)

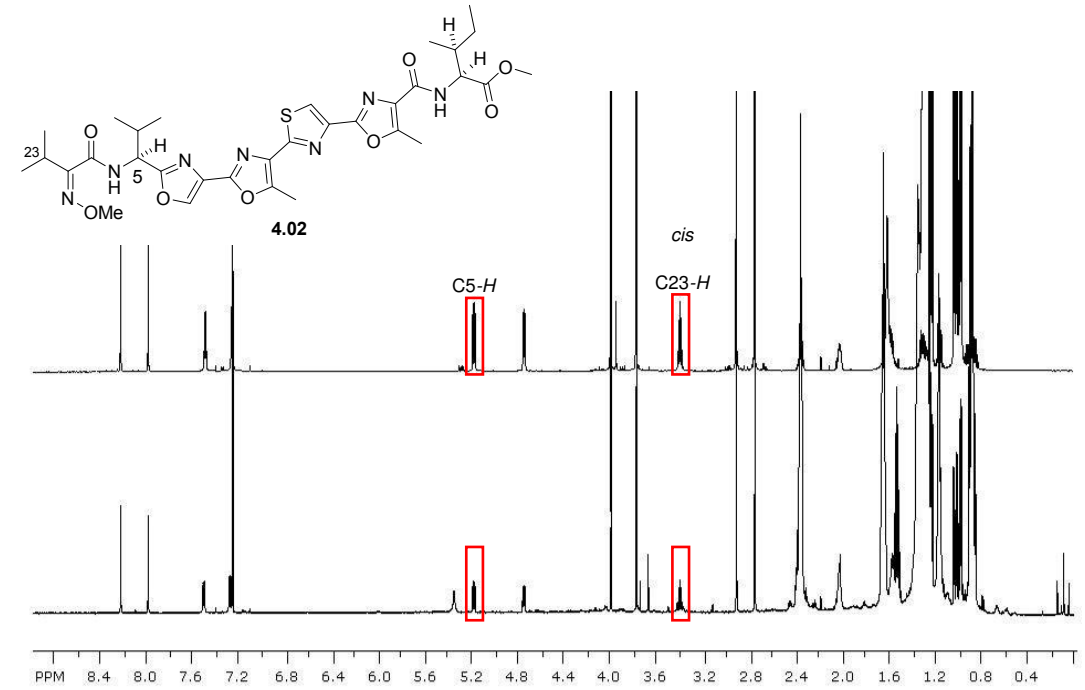
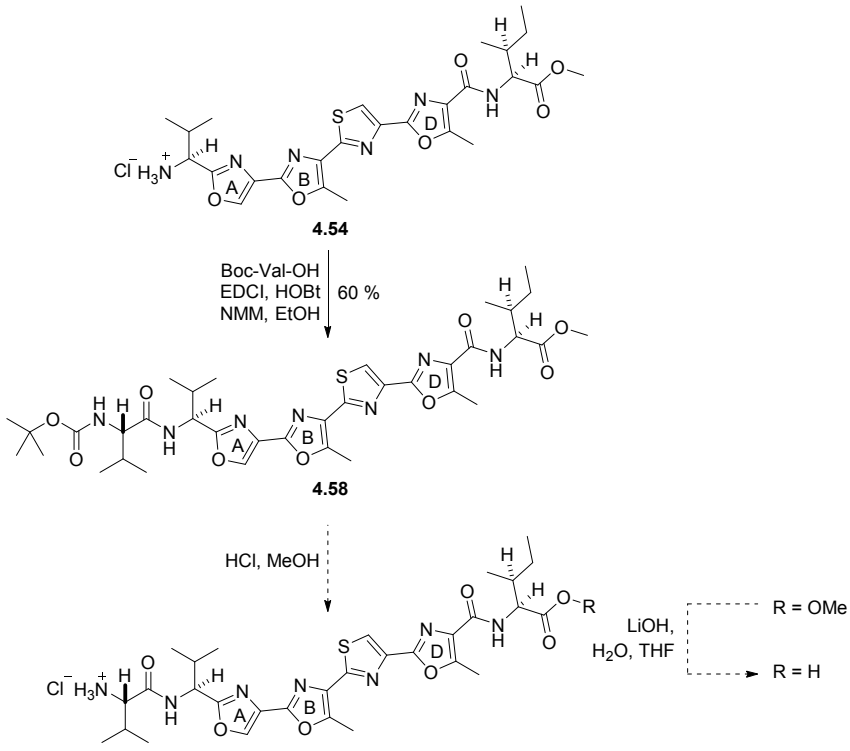


Figure 55: Top Spectrum: The ^{1}H NMR spectra showing the synthetic methoxyoxime product **4.02** as predominately the *cis* isomer. **Bottom Spectrum**: The ^{1}H NMR spectra showing the natural product, azolemycin B, from the bacterial extract as the *cis* isomer

4.7 SYNTHESIS OF A POSSIBLE BIOLOGICAL PRECURSOR OF AZOLEMYCIN A

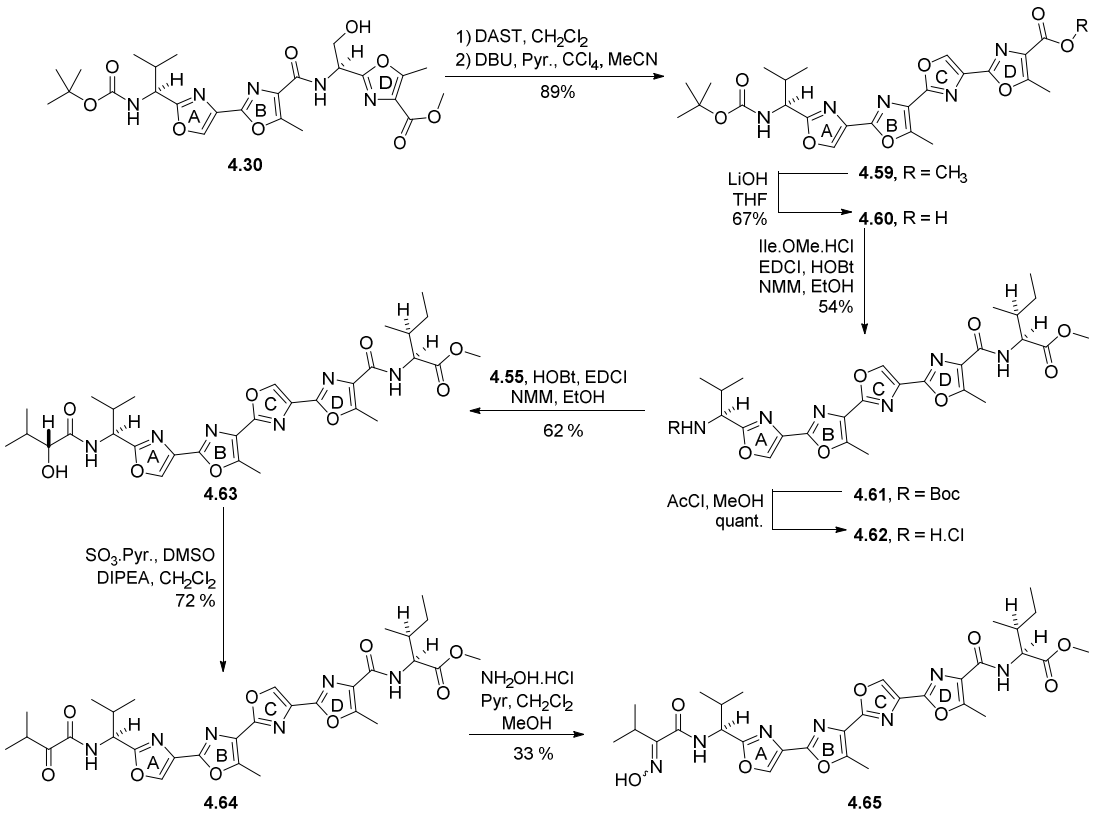
Following the successful total synthesis of azolemycins A and B, we were left with a reasonable amount of the precursor hexapeptide **4.54**. We therefore took the opportunity to synthesise precursor molecules that could be used to further clarify the proposed biosynthetic route (Scheme 48). It is currently uncertain whether the oxidation to the oxime or the methylation of the *C*-terminal acid occurs first. We therefore synthesised the reduced precursor **4.58** using the standard peptide coupling method. This could be selectively deprotected to give the free amine as both the methyl ester and the acid. Since the genes associated with the oxidation have been identified, the region associated with the flavin-dependent monooxygenase can be amplified. Challis and co-workers intend to achieve this and then attempt oxidation of both methyl ester and the acid. The enzyme may only oxidise one of these substrates, confirming the order of the methylation/oxidation.



Scheme 48: Synthesis of the protected precursor peptide, and the proposed route to the selectively deprotected products

4.8 SYNTHESIS OF A TETRA-OXAZOLE ANALOGUE OF AZOLEMYCIN A

With the compounds **4.01**, **4.02** and **4.58** synthesised, we had time in hand, along with sufficient quantities of building blocks **4.24** and **4.29**, to perform the synthesis of the tetra-oxazole analogue of azolemycin A, **4.65** (Scheme 49). This was achieved using the same reaction conditions as used for the synthesis of the natural products **4.01** and **4.02**, and progressed without incident to give 6 mg of oxazole analogue **4.65**. The only noteworthy moment in the synthesis was the comparative ease of the formation of the internal oxazole **C** compared to the *N*-terminal oxazole **A** in the bisoxazole fragment **4.35**. This supports our hypothesis that, while an adjacent electron withdrawing group at the 5-position is important for the oxidation of oxazolines, the reaction will still proceed in excellent yields if it benefits from conjugation with neighbouring azoles.



Scheme 49: The synthesis of the tetra-oxazole analogue of azolemycin A, **4.65**

4.8.1 Comparison of Azolemycin A and the Tetra-oxazole Analogue

The final oxime product **4.65** was obtained as predominately the *trans*-geometric isomer, in comparison to the thiazole-containing **4.02** which was isolated as predominately the *cis*-geometric isomer. Comparison of the ^{1}H NMR spectrum of **4.65** with the *trans*-isomer of azolemycin A from bacterial extract show relatively good correlation for most of the peaks, with the only significant difference being observed for the proton at C32. For the thiazole containing natural product, this peak occurs around 0.3 ppm downfield from the corresponding peak for the oxazole containing **4.65** (**Figure 56**).

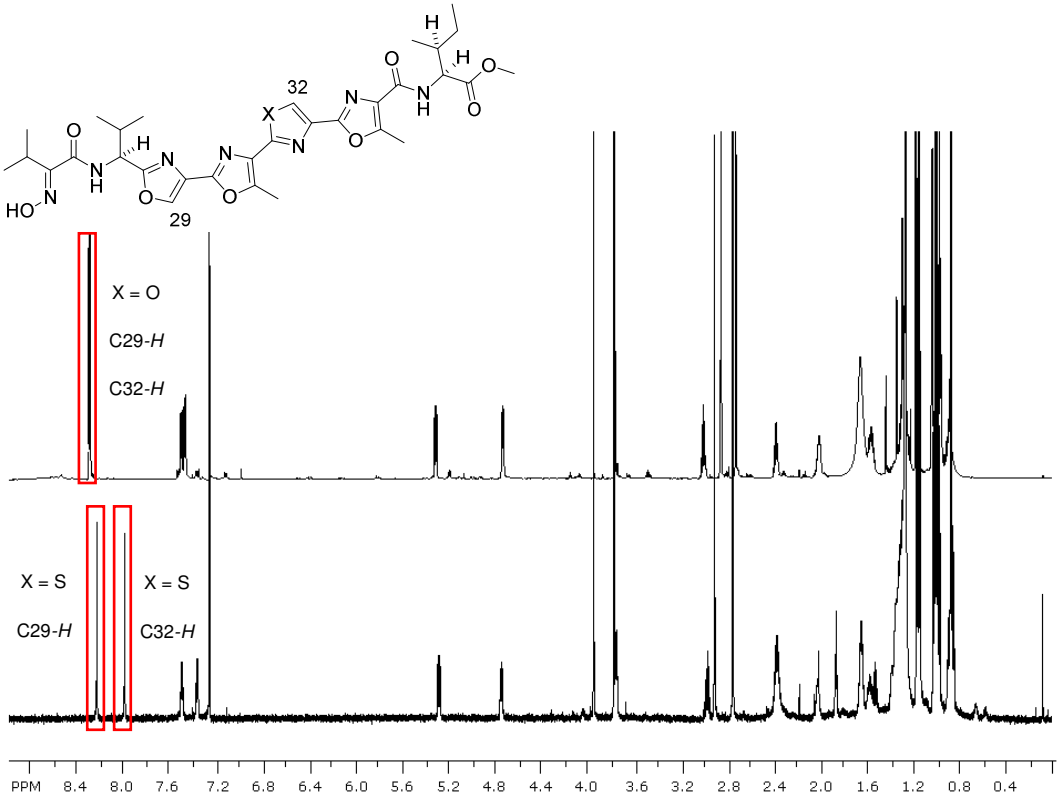


Figure 56: Top Spectrum: The ^{1}H NMR spectra showing the synthetic tetraoxazole product **4.65** as predominately the *trans* isomer, highlighting the peaks for C29-*H* and C32-*H*. **Bottom Spectrum:** The ^{1}H NMR spectra showing the natural product azolemycin A from bacterial extract as the *trans* isomer with the same peaks highlighted

Unfortunately, there is no ^{13}C spectrum of the *cis*-isomer of the biological extract of azolemycin A for comparison. However, comparison of the ^{13}C NMR spectra of the tetraoxazole **4.65** with the equivalent synthesized product **4.01** showed some

distinctive differences. As well as the obvious shift of the peak corresponding to *C*32-H from 120.2 ppm in **4.01** to 138.31 ppm in **4.65**, the peaks corresponding to carbons of azole **C**, and those immediately surrounding it, were shifted significantly (Figure 57). Some of these differences are highlighted below. One that has potentially useful implications for structural assignment of related oxazole and thiazole compounds is the difference in shift observed for the peak corresponding to *C11*, on the oxazole neighbouring azole **C**. In the thiazole-containing **4.01** the peak coreespponding to *C11* occurs at 130.9 ppm, but in the oxazole-containing **4.65** the *C11* peak appears at 125.7 ppm. It would be interesting to see if this relatively large difference in chemical shift between the oxazole and thiazole containing compounds is general within polyazole molecules, and if it would allow the identification of the relative positionings of oxazoles and thiazoles in a linked system.

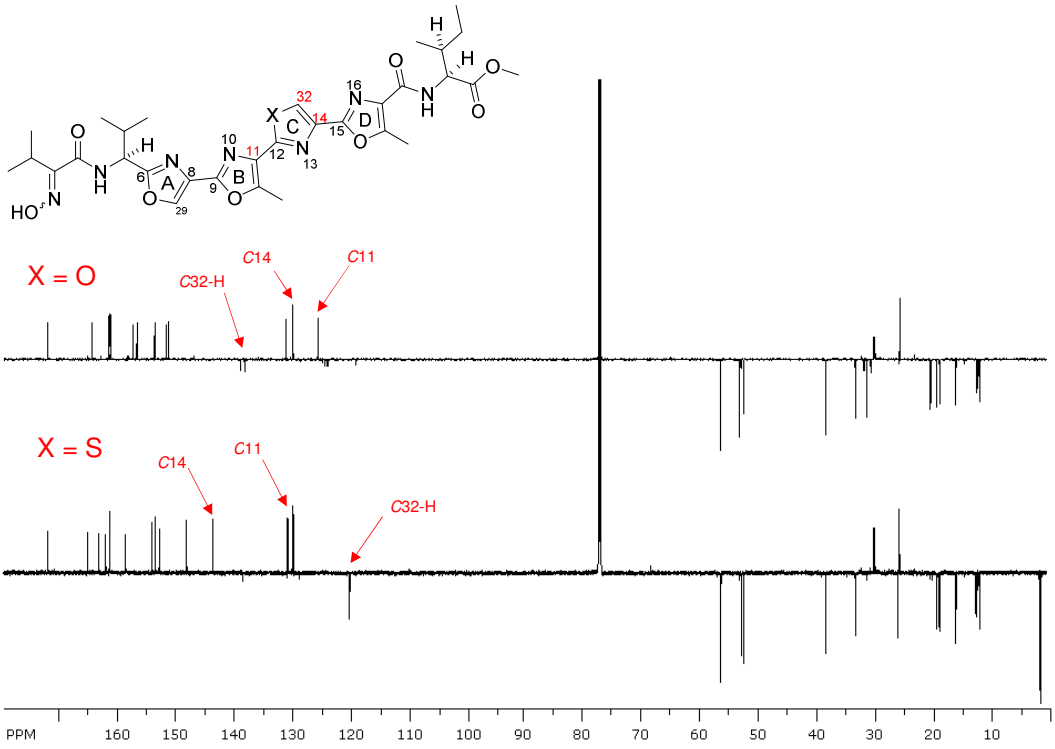


Figure 57: Top Spectrum: The ^{13}C NMR spectra showing the synthetic tetraoxazole product **4.65,** highlighting the peaks for *C*11-H, *C*14-H and *C*32-H. **Bottom Spectrum:** The ^{13}C NMR spectra showing the synthetic azolemycin A **4.01** (bottom spectrum) with the same carbons highlighted

4.9 REFERENCES

1. W. L. Kelly and C. A. Townsend, *J. Am. Chem. Soc.*, 2002, **124**, 8186-8187.
2. R. Winkler and C. Hertweck, *Angew. Chem., Int. Ed.*, 2005, **44**, 4083-4087.
3. S. Kirner, P. E. Hammer, D. S. Hill, A. Altmann, I. Fischer, L. J. Weislo, M. Lanahan, K.-H. van Pée and J. M. Ligon, *J. Bacteriol.*, 1998, **180**, 1939-1943.
4. V. S. C. Yeh, *Tetrahedron*, 2004, **60**, 11995-12042.
5. I. J. Turchi and M. J. S. Dewar, *Chem. Rev. (Washington, DC, U. S.)*, 1975, **75**, 389-437.
6. I. J. Turchi, *Ind. Eng. Chem. Prod. Res. Dev.*, 1981, **20**, 32-76.
7. K. Pfister, C. A. Robinson, A. C. Shabica and M. Tishler, *J. Am. Chem. Soc.*, 1949, **71**, 1101-1105.
8. A. F. Wagner, *J. Am. Chem. Soc.*, 1957, **79**, 3240-3245.
9. R. N. Boyd and R. H. Hansen, *J. Am. Chem. Soc.*, 1953, **75**, 5896-5897.
10. R. N. Misra, B. R. Brown, P. M. Sher, M. M. Patel, S. E. Hall, W. C. Han, J. C. Barrish, O. Kocy and D. N. Harris, *J. Med. Chem.*, 1993, **36**, 1401-1417.
11. P. Wipf and C. P. Miller, *Tetrahedron Lett.*, 1992, **33**, 6267-6270.
12. G. M. Atkins and E. M. Burgess, *J. Am. Chem. Soc.*, 1968, **90**, 4744-4745.
13. P. Wipf and C. P. Miller, *J. Org. Chem.*, 1993, **58**, 1575-1578.
14. P. Wipf and C. P. Miller, *Tetrahedron Lett.*, 1992, **33**, 907-910.
15. A. J. Phillips, Y. Uto, P. Wipf, M. J. Reno and D. R. Williams, *Org. Lett.*, 2000, **2**, 1165-1168.
16. D. R. Williams, P. D. Lowder, Y.-G. Gu and D. A. Brooks, *Tetrahedron Lett.*, 1997, **38**, 331-334.
17. J. C. Barrish, J. Singh, S. H. Spergel, W. C. Han, T. P. Kissick, D. R. Kronenthal and R. H. Mueller, *J. Org. Chem.*, 1993, **58**, 4494-4496.
18. T. Aoyama, N. Sonoda, M. Yamauchi, K. Toriyama, M. Anzai, A. Ando and T. Shioiri, *Synlett*, 1998, **1998**, 35-36.

19. A. Padwa, J. K. Rasmussen and A. Tremper, *J. Am. Chem. Soc.*, 1976, **98**, 2605-2614.

20. D. L. Evans, D. K. Minster, U. Jordis, S. M. Hecht, A. L. Mazzu and A. I. Meyers, *J. Org. Chem.*, 1979, **44**, 497-501.

21. J. R. Parikh and W. v. E. Doering, *J. Am. Chem. Soc.*, 1967, **89**, 5505-5507.

22. P. Wipf, A. Cunningham, R. L. Rice and J. S. Lazo, *Bioorg. Med. Chem.*, 1997, **5**, 165-177.

23. P. Wipf and S. Venkatraman, *J. Org. Chem.*, 1995, **60**, 7224-7229.

24. J.-M. Delacotte, H. Galons, D. Schott and J.-L. Morgat, *Synth. Commun.*, 1992, **22**, 3075-3079.

25. R. Robinson, *J. Chem. Soc., Trans.*, 1909, **95**, 2167-2174.

26. S. Gabriel, *Ber. Dtsch. Chem. Ges.*, 1910, **43**, 1283-1287.

27. M. C. Bagley, K. E. Bashford, C. L. Hesketh and C. J. Moody, *J. Am. Chem. Soc.*, 2000, **122**, 3301-3313.

28. T. Fukuyama and L. Xu, *J. Am. Chem. Soc.*, 1993, **115**, 8449-8450.

29. R. H. Wiley, *J. Org. Chem.*, 1947, **12**, 43-46.

30. R. C. Cookson and G. F. H. Green, *J. Chem. Soc.*, 1952, 827-833.

31. J. Lister and R. Robinson, *J. Chem. Soc., Trans.*, 1912, **101**, 1297-1315.

32. J. Deeley, A. Bertram and G. Pattenden, *Org. Biomol. Chem.*, 2008, **6**, 1994-2010.

33. J. R. Lewis, *Nat. Prod. Rep.*, 2002, **19**, 223-258.

34. Z. Jin, Z. Li and R. Huang, *Nat. Prod. Rep.*, 2002, **19**, 454-476.

35. Z. Jin, *Nat. Prod. Rep.*, 2013, **30**, 869-915.

36. D. Hernández, E. Riego, F. Albericio and M. Álvarez, *Eur. J. Org. Chem.*, 2008, **2008**, 3389-3396.

37. E. Riego, D. Hernández, F. Albericio and M. lvarez, *Synthesis*, 2005, **2005**, 1907-1922.

38. T. Ichiba, W. Y. Yoshida, P. J. Scheuer, T. Higa and D. G. Gravalos, *J. Am. Chem. Soc.*, 1991, **113**, 3173-3174.

39. P. Wipf and S. Venkatraman, *J. Org. Chem.*, 1996, **61**, 6517-6522.

40. A. Nagatsu, H. Kajitani and J. Sakakibara, *Tetrahedron Lett.*, 1995, **36**, 4097-4100.

41. H. Umezawa, *Prog. Biochem. Pharmacol.*, 1976, **11**, 18-27.

42. S. M. Hecht, *J. Nat. Prod.*, 1999, **63**, 158-168.

43. K. Gerth, H. Irschik, H. Reichenbach and W. Trowitzsch, *J. Antibiot.*, 1980, **33**, 1474-1479.

44. W. Kohl, B. Witte, G. Höfle, B. Kunze, H. Reichenbach, V. Wray and D. Schomburg, *Liebigs Ann. Chem.*, 1985, **1985**, 2088-2097.

45. P. Wipf and S. Lim, *J. Am. Chem. Soc.*, 1995, **117**, 558-559.

46. P. Wipf and C. P. Miller, *J. Org. Chem.*, 1993, **58**, 3604-3606.

47. D. R. Williams, D. A. Brooks and M. A. Berliner, *J. Am. Chem. Soc.*, 1999, **121**, 4924-4925.

48. F. Yokokawa, T. Asano and T. Shioiri, *Org. Lett.*, 2000, **2**, 4169-4172.

49. E. J. Zylstra, M. W. L. She, W. A. Salamant and J. W. Leahy, *Synlett*, 2007, **2007**, 0623-0627.

50. D. A. McGowan, U. Jordis, D. K. Minster and S. M. Hecht, *J. Am. Chem. Soc.*, 1977, **99**, 8078-8079.

51. B. J. Martin, J. M. Clough, G. Pattenden and I. R. Waldron, *Tetrahedron Lett.*, 1993, **34**, 5151-5154.

52. E. Aguilar and A. I. Meyers, *Tetrahedron Lett.*, 1994, **35**, 2473-2476.

53. E. Aguilar and A. I. Meyers, *Tetrahedron Lett.*, 1994, **35**, 2477-2480.

54. S. V. Downing, E. Aguilar and A. I. Meyers, *J. Org. Chem.*, 1999, **64**, 826-831.

55. K. C. Nicolaou, B. S. Safina, M. Zak, S. H. Lee, M. Nevalainen, M. Bella, A. A. Estrada, C. Funke, F. J. Zécri and S. Bulat, *J. Am. Chem. Soc.*, 2005, **127**, 11159-11175.

56. K. Shin-ya, K. Wierzba, K.-i. Matsuo, T. Ohtani, Y. Yamada, K. Furihata, Y. Hayakawa and H. Seto, *J. Am. Chem. Soc.*, 2001, **123**, 1262-1263.

57. M.-Y. Kim, H. Vankayalapati, K. Shin-ya, K. Wierzba and L. H. Hurley, *J. Am. Chem. Soc.*, 2002, **124**, 2098-2099.

58. T. Doi, M. Yoshida, K. Shin-ya and T. Takahashi, *Org. Lett.*, 2006, **8**, 4165-4167.

59. T. Doi, K. Shibata, M. Yoshida, M. Takagi, M. Tera, K. Nagasawa, K. Shin-ya and T. Takahashi, *Org. Biomol. Chem.*, 2011, **9**, 387-393.

60. J. Linder, T. P. Garner, H. E. L. Williams, M. S. Searle and C. J. Moody, *J. Am. Chem. Soc.*, 2010, **133**, 1044-1051.

61. M. C. Bagley, C. J. Moody and A. G. Pepper, *Tetrahedron Lett.*, 2000, **41**, 6901-6904.

62. M. C. Bagley, S. L. Hind and C. J. Moody, *Tetrahedron Lett.*, 2000, **41**, 6897-6900.

63. R. A. Hughes, S. P. Thompson, L. Alcaraz and C. J. Moody, *J. Am. Chem. Soc.*, 2005, **127**, 15644-15651.

64. K.-y. Sohda, K. Nagai, T. Yamori, K.-i. Suzuki and A. Tanaka, *J. Antibiot.*, 2005, **58**, 27-31.

65. D. Hernández, G. Vilar, E. Riego, L. M. Cañedo, C. Cuevas, F. Albericio and M. Álvarez, *Org. Lett.*, 2007, **9**, 809-811.

66. K.-y. Sohda, M. Hiramoto, K.-i. Suzumura, Y. Takebayashi, K.-i. Suzuki and A. Tanaka, *J. Antibiot.*, 2005, **58**, 32-36.

67. X.-H. Jian, H.-X. Pan, T.-T. Ning, Y.-Y. Shi, Y.-S. Chen, Y. Li, X.-W. Zeng, J. Xu and G.-L. Tang, *ACS Chem. Biol.*, 2012, **7**, 646-651.

68. G. Tojo and M. I. Fernández, *Oxidation of alcohols to aldehydes and ketones: a guide to current common practice*, Springer, 2006.

69. K. B. Sharpless, W. Amberg, Y. L. Bennani, G. A. Crispino, J. Hartung, K. S. Jeong, H. L. Kwong, K. Morikawa and Z. M. Wang, *J. Org. Chem.*, 1992, **57**, 2768-2771.

70. S. B. King and K. B. Sharpless, *Tetrahedron Lett.*, 1994, **35**, 5611-5612.

71. P. Zarantonello, C. P. Leslie, R. Ferritto and W. M. Kazmierski, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 561-565.

72. G. Videnov, D. Kaiser, C. Kempter and G. Jung, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1503-1506.

73. L. M. Martin and B.-H. Hu, *Tetrahedron Lett.*, 1999, **40**, 7951-7953.

74. G. T. K. Panse, S. K, *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.*

, 1989, **28**, 793-795.

75. S. Nordhoff, S. Bulat, S. Cerezo-Gálvez, O. Hill, B. Hoffmann-Enger, M. López-Canet, C. Rosenbaum, C. Rummey, M. Thiemann, V. G. Matassa, P. J. Edwards and A. Feurer, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 6340-6345.

76. D. C. Palmer, *The Chemistry of Heterocyclic Compounds, Oxazoles: Synthesis, Reactions, and Spectroscopy*, Wiley, 2004.

77. L. A. Carpino, *J. Org. Chem.*, 1970, **35**, 3971-3972.

78. A. J. Fatiadi, *Synthesis*, 1976, **1976**, 65-104.

79. S. P. Korshunov and I. V. Leontii, *Russ. Chem. Rev.*, 1966, **35**, 942.

80. R. J. Gritter and T. J. Wallace, *J. Org. Chem.*, 1959, **24**, 1051-1056.

81. M. Harfenist, A. Bavley and W. A. Lazier, *J. Org. Chem.*, 1954, **19**, 1608-1616.

82. J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen and T. Walker, *J. Chem. Soc.*, 1952, 1094-1111.

83. C. L. Angyal, R. J. W. Le Fevre, Y. Hirshberg, E. H. Frei, E. Fischer, E. C. Bate-Smith, T. Swain, C. W. L. Bevan, R. F. Hudson, O. Mancera, G. Rosenkranz and F. Sondheimer, *J. Chem. Soc.*, 1953, 2181-2191.

84. S. Ball, T. W. Goodwin and R. A. Morton, *Biochem. J.*, 1948, **42**, 516-523.

85. R. S. Varma, R. K. Saini and R. Dahiya, *Tetrahedron Lett.*, 1997, **38**, 7823-7824.

86. R. Stavrescu, T. Kimura, M. Fujita, M. Vinatoru and T. Ando, *Synth. Commun.*, 1999, **29**, 1719-1726.

87. I. M. Goldman, *J. Org. Chem.*, 1969, **34**, 1979-1981.

88. J. D. Miller and R.-Y. Wan, *Hydrometallurgy*, 1983, **10**, 219-242.

4.10 EXPERIMENTAL

All the reagents and solvents used were purchased from the Sigma-Aldrich, Alfa-Aesar, TCI, Bachem or Fluorochem Chemical Company and were used as received unless stated otherwise. pH 2 buffer was made as a solution of 0.75 M of Na_2SO_4 and 0.25 M H_2SO_4 in H_2O.

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AVII-700 MHz, AVIII-600 MHz, DRX-500 MHz or DPX-400 MHz Fourier transform spectrometer at room temperature unless stated otherwise. Chemical shifts are quoted in parts per million (ppm) downfield from tetramethylsilane. Solvents were used as an internal standard when assigning NMR spectra (δ_H: $CDCl_3$ 7.26 ppm, CD_3OD 3.31 ppm, DMSO-d_6, 2.50 ppm, D_2O 4.79 ppm; δ_C: $CDCl_3$ 77.1 ppm, CD_3OD 49.0 ppm, DMSO-d_6, 39.5 ppm). Coupling constants (*J*) are quoted in Hertz (Hz) and are rounded to the nearest 0.5 Hz. Abbreviations used in the descriptions of spectra are as follows; s = singlet, d = doublet, t = triplet, q = quartet, quin. = quintet, sept. = septet, oct. = octet, m = multiplet, br = broad, i = ipso, o = ortho, m = meta, p = para, ax. = axial and eq. = equatorial. ^{13} C-NMR spectra were recorded with broadband proton decoupling and spectra were assigned on the basis of COSY, PENDANT, HMQC and HMBC spectra. In aromatic characterisations, the ipso carbon is taken to be the carbon bonded to the group with the highest molecular weight

Infrared spectra were recorded on a Nicolet Avatar 320 FT-IR spectrophotometer using EZ OMNIC software package 1, Bruker ALPHA Platinum ATR spectrophotometer or Perkin ELMER Spectrum 100 FT-IR spectrophotometer using OPUS software and are quoted in wavenumber (cm^{-1}).

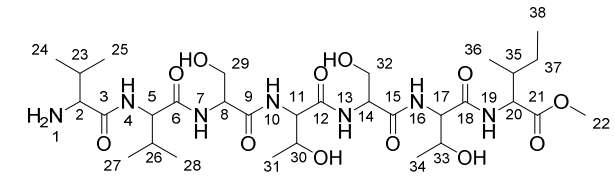
Optical rotations were recorded on an Optical Activity Ltd. AA-1000 millidegree auto-ranging polarimeter (using the sodium D line; 589 nm) and $[\alpha]_D$s are given in units of 10^{-1}deg cm^2 g^{-1}. The samples were made using spectroscopic grade MeOH, $CHCl_3$ or H_2O.

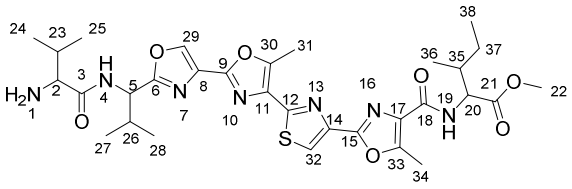
ESI mass spectra were obtained on a Bruker Esquire 2000 mass spectrometer or an Agilent 6130B single Quad (ESI)**.** HR ESI spectra were obtained by Dr Lijiang Song, Mr Philip Aston or Dr Rebecca Wills using a Bruker micro-TOF ESI attached to a time of flight (TOF) analyser.

Melting points for solid crystalline products were determined on a Stuart Scientific SMP10 Digital Melting Point Apparatus, with three runs of each compound, and a range given in ˚C rounded to the nearest degree. They are uncorrected. CHN elemental analyses were carried out by Warwick Analytical Services.

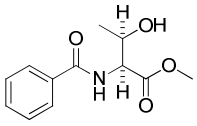
Thin Layer Chromatography (TLC) was performed using silica (0.25 mm) coated aluminium plates.

Numbering system





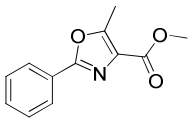
***N*-Benzoyl-L-threonine methyl ester 4.03**



Acetyl chloride (15.6 mL, 200 mmol) was added dropwise with stirring to MeOH (100 mL) at 0 - 5°C. L-Threonine (6.54 g, 54.9 mmol) was added and the resulting solution heated to reflux from 2 hours. The reaction mixture was concentrated *in vacuo* to give L-threonine methyl ester hydrochloride which was used without further purification. The resulting oil was dissolved in CH_2Cl_2 (160 mL) and triethylamine (17.4 mL, 125 mmol) and cooled to 0 °C with stirring. Benzoyl chloride (5.8 mL, 49.9 mmol) was added dropwise and the reaction mixture allowed to reach room temperature over 18 hours. After quenching with saturated sodium hydrogen carbonate solution (100 mL), the mixture was separated and the aqueous phase extracted with CH_2Cl_2 (2 x 100 mL). The combined organic extracts were concentrated *in vacuo* and the resulting yellow oil partitioned between EtOAc (100 mL) and pH 2 buffer (100 mL). The organic phase was further washed with pH 2 buffer (100 mL), water (100 mL), saturated aqueous $NaHCO_3$ solution (2 x 100 mL) and saturated aqueous NaCl solution (100 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the crude product as a bright yellow oil. Recrystallisation (EtOAc:petroleum ether) afforded **4.03** as a fluffy white solid (8.03 g, 33.8 mmol, 62 %); mp. 96 - 97 °C (lit.[1] 97 - 98 °C); $[\alpha]_D^{25}$ +18.4 (c = 1.02, $CHCl_3$), (lit.[2] $[\alpha]_D^{20}$

+22.6 (c = 1.0 in $CHCl_3$)); υ_{max}/cm^{-1} 3425 (O-H), 3348 (N-H), 1741 (ester C=O), 1640 (amide C=O), 1521 (N-H); δ_H (400 MHz, $CDCl_3$) 7.91 - 7.78 (2H, m, *ortho* C-*H*), 7.55 - 7.48 (1H, m, *para* C-*H*), 7.46 - 7.41 (2H, m, *meta* C-*H*), 7.04 (1H, d, *J* 8.5 Hz, CHN*H*), 4.82 (1H, dd, *J* 8.5, 2.5 Hz, C*H*NH), 4.50 - 4.40 (1H, m, C*H*OH), 3.78 (3H, s, OCH_3), 2.77 (1H, d, *J* 4.0 Hz, CHO*H*), 1.28 (3H, d, *J* 6.5 Hz, $CHCH_3$); δ_C (100 MHz, $CDCl_3$) 171.7 (*C*ONH), 168.0 (CO_2Me), 133.7 (*ipso C*), 132.0 (*para* C-H), 128.6 (*ortho C*-H), 127.2 (*meta C*-H), 68.2 (*C*HOH), 57.7 (*C*HNH), 52.7 (OCH_3), 20.0 ($CHCH_3$); *m/z* (ESI+) 260.1 ([M+Na], 100%); HR-ESIMS: calculated for $C_{12}H_{15}NO_4Na$: 260.0893, found 260.0888 $[M+Na]^+$. The data are consistent with that previously reported.[3]

5-Methyl-2-phenyl-oxazole-4-carboxylic acid methyl ester 4.04

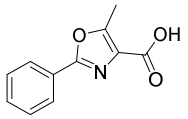


Method modified from literature procedure by Pattenden *et al.*[4] (Diethylamino)sulfur trifluoride (2.4 mL, 18.0 mmol) was added dropwise to a stirred solution of ester **4.03** (3.6 g, 15.0 mmol) in dry CH_2Cl_2 (150 mL) at - 78°C under N_2. The mixture was stirred at – 78 °C for 1.5 hours, then allowed to reach room temperature and stirred for a further 15 minutes. The reaction was quenched by addition of saturated sodium bicarbonate solution (80 mL) and separated. The organic phase was dried and concentrated *in vacuo* to give the crude oxazolidine as a clear oil, which was used immediately without further purification.

Bromotrichloromethane (4.4 mL, 46 mmol) was added to a stirred solution of the crude oxazolidine in dry CH_2Cl_2 (150 mL) at 0°C under N_2 and the mixture was stirred at 0°C for 5 minutes. 1, 8-Diazabicyclo[5.4.0]undec-7-ene (6.9 mL, 46 mmol) was added dropwise and the mixture allowed to warm to room temperature overnight. The reaction was quenched with 10% aqueous citric acid (80 mL) and the phases were separated. The aqueous phase was re-extracted with dichloromethane (2

x 80 mL) and the combined organic extracts concentrated *in vacuo* to give a dark brown residue. This was partitioned between EtOAc (100 mL) and 10% aqueous citric acid (80 mL) and the separated organic extract washed with saturated $NaHCO_3$ solution (80 mL), saturated aqueous NaCl solution (80 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica chromatography (9:1 hexanes:EtOAc, Rf. = 0.33) to give oxazole **4.04** as a white crystalline solid (2.10 g, 9.7 mmol, 64 % over 2 steps); m.p. 94 - 95°C (lit.[5] 93 - 94°C); υ_{max} /cm^{-1} (neat) 2960 (aromatic C-H), 1722 (C=O); δ_H (400 MHz, $CDCl_3$) 8.14 - 8.02 (2H, m, *ortho* C-*H*), 7.52 - 7.42 (3H, m, *meta, para* C-*H*), 3.96 (3H, s, OCH_3), 2.72 (3H, s, CCH_3); δ_C (100 MHz, $CDCl_3$) 162.8, 159.6, 156.4 (3 x quaternary *C*), 130.7 (*para C*-H), 128.7 (*meta C*-H), 128.5 (quaternary *C*), 126.5 (*ortho C-H*), 52.0 (O*C*H$_3$), 12.1 (C*C*H$_3$). One carbon unaccounted for; *m/z* (ESI+) 240.0 ([M+Na], 100%), 218.0 ([M+H], 54%); HR-ESIMS: calculated for $C_{12}H_{11}NO_3Na$: 240.0631, found 240.0628 $[M+Na]^+$. The data are consistent with that previously reported.[6]

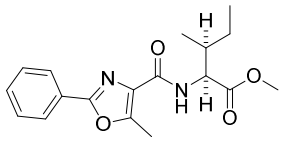
5-Methyl-2-phenyl-oxazole-4-carboxylic acid 4.05



Methyl ester **4.04** (2.04 g, 9.4 mmol) was dissolved in a mixture of 3 M aqueous NaOH solution (20 mL, 60 mmol) and MeOH (32 mL) and heated to 45 °C for 2 hours. The reaction was acidified to pH 1 with 12 M aqueous hydrochloric acid solution and extracted with diethyl ether (3 x 100 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give carboxylic acid **4.05** as an off white crystalline solid (1.30 g, 6.4 mmol, 68 %); m.p. 182 - 183 °C (lit.[7] 181.5 - 183 °C); υ_{max}/cm^{-1} 2970 (O-H), 1717 (C=O); δ_H (400 MHz, DMSO-d_6) 13.36 - 12.73 (1H, m, CO_2H), 8.15 - 7.89 (2H, m, *ortho* C-*H*), 7.68 - 7.25 (3H, m, *meta* and *para* C-*H*), 2.65 (3H, s, CCH_3); δ_C (100 MHz, DMSO-d_6) 163.1, 158.5, 156.2 (3 x quaternary *C*), 131.0 (*para C*-H), 129.3 (*meta C*-H), 128.8, 126.3 (2 x quaternary *C*), 126.0 (*ortho C*-H), 12.0 (C*C*H$_3$); *m/z* (ESI+) 204.0 ([M+H], 100%),

226.0 ([M+Na], 30%); HR-ESIMS: calculated for $C_{11}H_9NO_3Na$: 226.0475, found 226.0478 $[M+Na]^+$. The data are consistent with that previously reported.[6]

_N_-(5-Methyl-2-phenyl-oxazole-4-carboxy)-L-isoleucine methyl ester 4.06

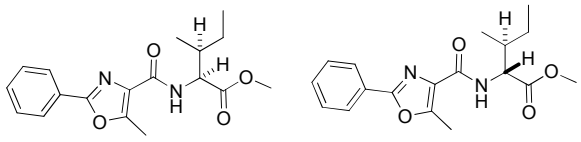


Acetyl chloride (16.3 mL, 229 mmol) was added dropwise with stirring to MeOH at 0°C. L-Isoleucine (5.00 g, 38.1 mmol) was added and the mixture was heated to reflux overnight. The reaction mixture was concentrated *in vacuo* and the crude L-isoleucine methyl ester hydrochloride was used without further purification.

A solution of L-isoleucine methyl ester hydrochloride (1.08 g, 6.40 mmol) and triethylamine (0.9 mL, 6.4 mmol) in CH_2Cl_2 (20 mL) was added to a stirred and cooled suspension of HATU (2.4 g, 6.4 mmol) and carboxylic acid **4.05** (1.3 g, 6.4 mmol) in CH_2Cl_2 (20 mL) at 0 - 5°C. The reaction mixture was stirred at 0 -5 °C for 15 minutes, and triethylamine (1.8 mL, 12.8 mmol) was added dropwise. The reaction mixture was allowed to reach room temperature overnight. To the resulting yellow solution was added pH 2 buffer (100 mL), and the separated aqueous phase was further extracted in CH_2Cl_2 (2 x 50 mL). The combined organic residues were concentrated *in vacuo* and the resulting yellow residue partitioned between pH 2 buffer (100 mL) and toluene (100 mL). The separated organic phase was washed with water (2 x 50 mL), saturated aqueous $NaHCO_3$ solution (50 mL) and saturated aqueous NaCl solution (50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The methyl ester **4.06** was obtained by silica chromatography (2% MeOH: CH_2Cl_2) as a colourless oil (0.8 g, 2.4 mmol, 38 %); $[\alpha]_D^{27}$ -23.2 (c = 1.1, $CHCl_3$); υ_{max}/cm^{-1} 2964 (N-H), 1740 (ester C=O), 1670 (amide C=O), 1580 (N-H); δ_H (600 MHz, $CDCl_3$) 8.26 - 7.94 (2H, m, *ortho* C-*H*), 7.53 (1H, d, *J* 8.5 Hz, CHN*H*), 7.49 - 7.41 (3H, m, *meta* and *para* C-*H*), 4.74 (1H, dd, *J* 9.0, 5.5 Hz, NHC*H*), 3.77 (3H, s, OCH_3), 2.71 (3H, s, CCH_3), 2.03 (1H, dqt, *J* 9.5, 7.0, 5.0 Hz, C*H*CH_3), 1.56 (1H, dqd, *J* 15.0, 7.0,

4.5 Hz, CHCH_2), 1.29 (1H, ddq, *J* 15.0, 9.5, 7.5 Hz, CHCH_2), 1.00 (3H, d, *J* 7.0 Hz, CHCH_3), 0.97 (3H, t, *J* 7.5 Hz, CH_2CH_3); δ_C (125 MHz, $CDCl_3$) 172.3 (CO_2Me), 161.8, 158.6, 153.2 (3 x quaternary *C*), 130.6 (Ar *C*-H), 129.9 (quaternary *C*), 128.7 (Ar *C*-H), 126.8 (quaternary *C*), 126.4 (Ar *C*-H), 56.0 (NH*C*H), 52.1 (OCH_3), 38.0 ($CHCH_3$), 25.2 (CH_2CH_3), 15.6 (CHCH_3), 11.8 (oxazole CH_3), 11.5 (CH_2CH_3); *m/z* (ESI+) 331.1 ([M+H], 100%), 353.1 ([M+Na], 85%); HR-ESIMS: calculated for $C_{18}H_{22}N_2O_4Na$: 353.1472, found 353.1479 $[M+Na]^+$.

(2*S*, 3*S*) and (2*R*, 3*S*)-2-(*N*-(5-Methyl-2-phenyl-oxazole-4-carboxyamino)-3-methyl-pentanoic acid methyl ester 4.07

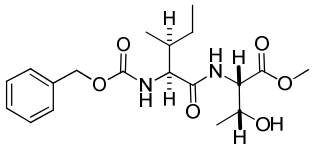


Method modified from literature procedure by du Vigneaud and Curtis.[8] A solution of methyl ester **4.06** (0.10 g, 0.29 mmol) in 3 M aqueous NaOH solution (6 mL, 18.2 mmol) and MeOH (5 mL) was heated to 45 °C overnight. The reaction was acidified to pH 1 with 12 M aqueous hydrochloric acid and extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give the acid as a white solid. This was dissolved in 3 M aqueous NaOH solution (0.5 mL, 1.8 mmol), THF (0.5 mL) and water (0.5 mL). Acetic anhydride was added (0.05 mL, 0.5 mmol) and the reaction mixture was heated to 65 °C for 3 days. The reaction was cooled to room temperature, acidified with pH 2 buffer (5 mL) and extracted with EtOAc (5 x 10 mL). The combined organics were dried over Na_2SO_4 and concentrated *in vacuo* to give the crude epimerisation product.

This was dissolved in a solution of acetyl chloride (0.43 mL, 6 mmol) in MeOH (2.5 mL) and heated to reflux for 18 hours. The reaction mixture was concentrated *in vacuo* to afford methyl ester **4.07** as a mix of diastereomers (brown oil, 0.096 g, 0.29 mmol, 97%, 50:50 mix of (2*S*, 3*S*) and (2*R*, 3*S*), calculated by ^{1}H NMR); υ_{max}/cm^{-1} 2962 (N-H), 1739 (ester C=O), 1649 (amide C=O), 1506 (N-H); δ_H (500

MHz, $CDCl_3$) 8.10 - 7.99 ((2*S,* 3*S*), 2H, m, *ortho* C-*H*; (2*R,* 3*S*), 2H, m, *ortho* C-*H*), 7.51 - 7.43 ((2*S,* 3*S*), 4H, m, *meta* and *para* C-*H*, N*H*CH; (2*R,* 3*S*), 4H, m, *meta* and *para* C-*H*, N*H*CH), 4.85 ((2*R,* 3*S*), 1H, dd, *J* 9.5, 4.5 Hz, NHC*H*), 4.74 ((2*S,* 3*S*), 1H, dd, *J* 9.0, 5.5 Hz, NHC*H*), 3.77 ((2*S,* 3*S*), 3H, s, OCH_3; (2*R,* 3*S*), 3H, s, OCH_3), 2.72 ((2*S,* 3*S*), 3H, s, CCH_3; (2*R,* 3*S*), 3H, s, CCH_3), 2.15 - 1.94 ((2*S,* 3*S*), 1H, m, C*H*CH_3; (2*R,* 3*S*), 1H, m, C*H*CH_3), 1.66 - 1.44 ((2*S,* 3*S*), 1H, m, CHCH_2; (2*R,* 3*S*), 1H, m, CHCH_2), 1.39 - 1.16 ((2*S,* 3*S*), 1H, m, CHCH_2; (2*R,* 3*S*), 1H, m, CHCH_2), 1.02 - 0.94 ((2*S,* 3*S*), 6H, m, CHCH_3, CH_2CH_3; (2*R,* 3*S*), 6H, m, CHCH_3, CH_2CH_3); δ_C (126MHz, $CDCl_3$) 172.7, 172.3 ((2*S,* 3*S*), *C*O_2Me; (2*R,* 3*S*), *C*O_2Me), 162.0 161.8, 158.6, 153.2 ((2*S,* 3*S*), 3 x quaternary *C*; (2*R,* 3*S*), 3 x quaternary *C*), 130.6 ((2*S,* 3*S*), Ar *C*-H; (2*R,* 3*S*), Ar *C*-H), 129.9 ((2*S,* 3*S*), quaternary *C*; (2*R,* 3*S*), quaternary *C*), 128.7 ((2*S,* 3*S*), Ar *C*-H; (2*R,* 3*S*), Ar *C*-H), 126.8 ((2*S,* 3*S*), quaternary *C*; (2*R,* 3*S*), quaternary *C*), 126.4 ((2*S,* 3*S*), Ar *C*-H; (2*R,* 3*S*), Ar *C*-H), 56.0, 55.0 ((2*S,* 3*S*), NH*C*H; (2*R,* 3*S*), NH*C*H), 52.2, 52.1 ((2*S,* 3*S*), O*C*H_3; (2*R,* 3*S*), O*C*H_3), 38.0, 37.8 ((2*S,* 3*S*), *C*HCH_3; (2*R,* 3*S*), *C*HCH_3), 26.3 ((2*S,* 3*S*), *C*H_2CH_3; (2*R,* 3*S*), *C*H_2CH_3), 15.6, 14.8 ((2*S,* 3*S*), CH*C*H_3; (2*R,* 3*S*), CH_2*C*H_3), 11.8 ((2*S,* 3*S*), oxazole *C*H_3; (2*R,* 3*S*), oxazole *C*H_3), 11.7, 11.5 ((2*S,* 3*S*), CH_2*C*H_3; (2*R,* 3*S*), CH_2*C*H_3); *m/z* (ESI+) 353.1 ([M+Na], 100%), 331.2 ([M+H], 82%); HR-ESIMS: calculated for $C_{18}H_{23}N_2O_4$: 331.1652, found 331.1659 $[M+H]^+$.

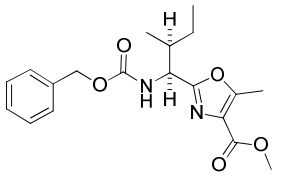
_N_-Carboxybenzyl-L-isoleucyl-L-threonine methyl ester 4.11



L-Threonine (5.00 g, 42.0 mmol) was added to a solution of acetyl chloride (18.0 mL, 253 mmol) in MeOH (100 mL), and the reaction mixture was heated to reflux for 5 hours. The reaction was cooled to room temperature and concentrated *in vacuo* to give the crude methyl ester hydrochloride as a sticky oil which was used without further purification.

A solution of *N*-carboxylbenzyl-L-isoleucine dicyclohexylammonium salt (17.1 g, 38.2 mmol) and HATU (14.5 g, 38.2 mmol) in CH_2Cl_2 (200 mL) was cooled to 0 - 5 °C and stirred for 15 minutes. To this solution was added a solution of the crude L-threonine methyl ester (42 mmol) and triethylamine (5.3 mL, 38.0 mmol) in CH_2Cl_2 (100 mL). The reaction mixture was stirred for a further 10 minutes before triethylamine (10.7 ml, 76.7 mmol) was added dropwise. The resulting yellow cloudy solution was allowed to reach room temperature overnight. pH 2 buffer (100 mL) was added and the reaction filtered. The filtercake was washed with CH_2Cl_2 (2 x 50 mL). The filtrate was separated, and the separated aqueous filtrate was further extracted with CH_2Cl_2 (3 x 100 mL). The combined organic extracts were concentrated *in vacuo* and the yellow oily solid obtained suspended in toluene: EtOAc (3:1, 200 mL). The suspension was filtered and the filtercake recrystallised (EtOAc: pet. ether) to give the dipeptide **4.17** as a white solid (6.70 g, 17.6 mmol, 46 %); m.p. 149 - 150 °C (lit.[9] 152 - 154 °C); $[\alpha]_D^{26}$ -19.7 (c = 1.1, MeOH) (lit.[9] $[\alpha]_D^{25}$ -20.5 (c = 3.34, MeOH); υ_{max}/cm^{-1} 3307 (N-H), 3272 (O-H), 1731 (ester C=O), 1688 (carbamate C=O), 1646 (amide C=O), 1536 (N-H); δ_H (400 MHz, DMSO-d_6) 7.89 (1H, d, *J* 8.0 Hz, CON*H*CH), 7.39 (1H, d, *J* 9.0 Hz, CO_2N*H*CH), 7.37 - 7.28 (5H, m, Ar C-*H*), 5.03 (2H, s, CH_2Ph), 4.98 (1 H, d, *J* 5.5 Hz, CHO*H*), 4.29 (1H, dd, *J* 8.5, 3.5 Hz, CONHC*H*), 4.12 (1H, qnd, *J* 6.5, 3.5 Hz, C*H*OH), 4.03 (1H, dd, *J* 9.0, 8.0 Hz, CO_2NHC*H*), 3.61 (3H, s, OCH_3) 1.82 – 1.68 (1H, m, C*H*CH_2), 1.43 (1H, dqd, *J* 15.0, 7.5, 3.5 Hz, CH_2CH_3), 1.33 – 1.09 (1H, m, CH_2CH_3), 1.06 (3H, d, *J* 6.5 Hz, CH(OH)CH_3), 0.86 (3H, d, *J* 7.0 Hz, CH(CH_2)CH_3), 0.81 (3H, t, *J* 7.5 Hz, CH_2CH_3); δ_C (100 MHz, DMSO-d6) 171.9, 171.0 (CO_2CH_3, NH*C*O), 156.0 (PhCH_2*C*O), 137.1 (*ipso C*-H), 128.3 (Ar *C*-H), 127.7 (*para C*-H), 127.6 (Ar *C*-H), 66.2 (*C*HOH), 65.3 (*C*H_2Ph), 59.1 (CO_2NH*C*H), 57.7 (CONH*C*H), 51.7 (O*C*H_3), 36.3 (*C*HCH_2), 24.3 (*C*H_2CH_3), 20.0 (CH(OH)*C*H_3), 15.2 (CH(CH_2)*C*H_3), 10.9 (*C*H_2CH_3); *m/z* (ESI+) 403.2 ([M+Na], 100%); HR-ESIMS: calculated for $C_{19}H_{29}N_2O_6$: 381.2020, found 381.2014 $[M+H]^+$. This compound has been previously reported, but without any spectroscopic data.[9]

Oxazole 4.12



Method modified from literature procedure by Doi *et al.*[10] A solution of *N*-carboxybenzyl-L-isoleucyl-L-threonine methyl ester **4.11** (1.00 g, 2.63 mmol) in dry CH_2Cl_2 (10 mL) was cooled to -20°C under nitrogen. Triethylamine (1.1 mL, 8.0 mmol) was added dropwise, followed by a suspension of SO_3.pyridine complex (1.27 g, 7.96 mmol) in dry DMSO (4 mL). The reaction mixture was allowed to warm to room temperature and stirred for 30 minutes to give a bright yellow solution. This was cooled to -20°C and quenched with saturated sodium hydrogen carbonate solution (5 mL). Upon warming to room temperature, the mixture was extracted with hexanes: EtOAc (75: 25, 2 x 10 mL). The combined organics were washed with saturated aqueous NaCl solution, dried over Na_2SO_4 and concentrated *in vacuo* to the crude product as a yellow white solid. The keto-ester was obtained by silica chromatography (50 % EtOAc: pet. ether) and used immediately. HR-ESIMS: calculated for $C_{19}H_{26}N_2O_6Na$: 401.1683, found 401.1681 $[M+Na]^+$.

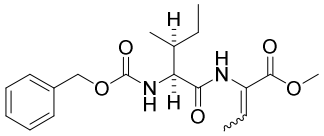
Iodine (0.83 g, 3.28 mmol) and triethylamine (1.15 mL, 8.20 mmol) were added sequentially to a stirred solution of triphenylphosphine (0.95 g, 3.60 mmol) in dry THF (15 mL) at room temperature under an atmosphere of nitrogen. The mixture was cooled to -78 °C and a solution of the ketone (0.62 g, 1.64 mmol) in dry THF (4 mL) was added dropwise to give a bright yellow suspension. The reaction mixture was allowed to warm to room temperature and stirred under nitrogen for a further 2.5 hours. The reaction mixture was concentrated *in vacuo* and partitioned between EtOAc (25 mL) and water (25 mL). The phases were separated and the aqueous phase extracted with EtOAc (2 x 25 mL). The combined organic phases were washed with aqueous hydrochloric acid solution (3M, 20 mL), saturated sodium hydrogen carbonate solution (20 mL), saturated aqueous NaCl solution (20 mL), dried over

Na_2SO_4 and concentrated *in vacuo*. The oxazole **4.12** was obtained by silica chromatography (10 % EtOAc: pet. ether to 25 % EtOAc: pet. ether) as a bright white solid (0.28 g, 0.78 mmol, 47 %); m.p. 103 - 104 °C; $[\alpha]_D^{27}$ -23.2 (c = 1.09, $CHCl_3$); υ_{max}/cm^{-1} 3306 (N-H), 1715 (ester C=O), 1686 (carbamate C=O), 1615 (amide C=O), 1539 (N-H); δ_H (400 MHz, $CDCl_3$) 7.37 - 7.14 (5H, m, Ar C-*H*), 5.55 (1H, d, *J* 9.5 Hz, N*H*CH), 5.10 - 4.97 (2H, m, PhCH_2), 4.80 (1H, dd, *J* 8.5, 6.5 Hz, NHC*H*), 3.83 (3H, s, OCH_3), 2.53 (3H, s, CCH_3), 1.96 - 1.82 (1H, m, C*H*CH_3), 1.50 - 1.38 (1H, m, CH_2CH_3), 1.18 - 1.04 (1H, m, CH_2CH_3), 0.88 - 0.78 (6H, m, CHCH_3, CH_2CH_3); δ_C (100 MHz, $CDCl_3$) 162.6, 161.7, 156.3, 155.9 (*C*O_2Me, *C*O_2Bn, 2 x oxazole *C*), 136.2 (*ipso C*), 128.5, 128.1, 128.0 (*ortho, meta* and *para C*H), 127.6 (oxazole *C*), 67.0 (PhCH_2), 53.8 (NH*C*H), 51.9 (O*C*H_3), 39.2 (*C*HCH_3), 25.0 (*C*H_2CH_3), 15.2 (CH*C*H_3), 12.0 (oxazole *C*H_3), 11.3 (CH_2*C*H_3); *m/z* (ESI+) 383.1 ([M+Na]+, 100.0%), 361.2 ([M+H]+, 28%); HR-ESIMS: calculated for $C_{19}H_{25}N_2O_5$: 361.1758, found 361.1761 $[M+H]^+$.

Oxazole **4.12** was also synthesised using (diethylamino)sulfur trifluoride. A solution of the dipeptide 4.11 (1.00 g, 2.63 mmol) in dry CH_2Cl_2 (25 mL) under nitrogen was cooled to -78 °C and (diethylamino)sulfur trifluoride (0.50 mL, 3.68 mmol) was added dropwise and the reaction mixture stirred at -78 °C for 1.5 hrs. The mixture was allowed to reach room temperature over 15 minutes before carefully quenching with saturated aqueous $NaHCO_3$. The mixture was stirred for 15 minutes and the phases were separated. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to give the crude oxazoline as a clear yellow oil. This was dissolved in dry CH_2Cl_2 (25 mL) under a nitrogen atmosphere and cooled to 0 °C. $BrCCl_3$ (0.32 mL, 3.23 mmol) was added dropwise and the reaction mixture was stirred for 5 minutes. 1, 8-Diazabicyclo[5.4.0]undec-7-ene (0.42 mmol, 3.02 mmol) was added and the reaction mixture was stirred to room temperature overnight. The reaction was quenched with 10% aqueous citric acid solution (20 mL) and the phases were separated. The aqueous phase was further extracted with CH_2Cl_2 (2 x 25 mL) and the

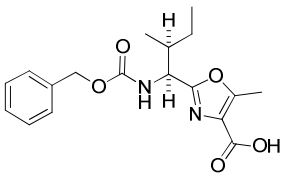
organic phases were combined and concentrated *in vacuo*. The residue was partitioned between EtOAc (30 mL) and 10% citric acid solution (30 mL). The phases were separated and the organic phase was washed with saturated aqueous $NaHCO_3$ solution (10 mL), saturated NaCl solution (10 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give a beige solid. The oxazole was obtained by silica chromatography (60 % pet. ether: EtOAc) as a white solid (0.30 g, 83.9 mmol, 32 %, Rf = 0.43). The characterization data was identical to that prepared in the previous method.

Alkene 4.13



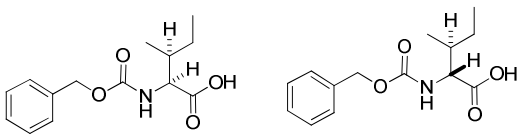
Also isolated was the elimination product **4.13** as a pale yellow solid (0.14 g, 39 mmol, 15 %, Rf = 0.30); m.p. 149 – 151 °C; υ_{max}/cm^{-1} 3289 (N-H), 1728 (ester C=O), 1685 (carbamate C=O), 1614 (amide C=O), 1534 (N-H); δ_H (400 MHz, $CDCl_3$) 7.43 - 7.30 (5H, m, Ar C-*H*), 7.23 (1H, br. s, N*H*C=C), 6.84 (1H, q, *J* 7.0 Hz, *H*(CH_3)C=C), 5.34 (1H, d, *J* 8.0 Hz, N*H*CH), 5.14 (2H, s, PhCH_2), 4.19 (1H, dd, *J* 8.0, 6.0 Hz, NHC*H*), 3.76 (3H, s, OCH_3), 2.04 - 1.91 (1H, m, C*H*CH_3), 1.76 (3H, d, *J* 7.0 Hz, H(CH_3)C=C), 1.62 - 1.50 (1H, m, C$H_2$$CH_3$), 1.20 (1H, ddq, *J* 13.5, 9.5, 7.0 Hz, C$H_2$$CH_3$), 1.02 (3H, d, *J* 6.5 Hz, CHCH_3), 0.94 (3H, t, *J* 7.5 Hz, CH_2CH_3); δ_C (175 MHz, $CDCl_3$) 169.6 (*C*ONH), 164.7 (*C*O_2Me), 156.4 (*C*OCH_2Ph), 136.2 (*ipso C*), 134.7 (H(CH_3)*C*=C), 128.6 (Ar *C*H), 128.3 (*para C*H), 128.1 (Ar *C*H), 125.6 (H(CH_3)C=*C*), 67.2 (*C*H_2Ph), 60.0 (NH*C*H), 52.4 (O*C*H_3), 37.3 (*C*HCH_3), 24.8 (*C*$H_2$$CH_3$), 15.5 (CH*C*$H_3$), 14.8 (H(*C*$H_3$)C=C), 11.5 ($CH_2$*C*$H_3$); *m/z* (ESI+) 385.1 ([M+Na]+), 363.1 ([M+H]+); HR-ESIMS: calculated for $C_{19}H_{26}N_2O_5Na$: 385.1738, found 385.1744 $[M+Na]^+$.

Oxazole Carboxylic Acid 4.14



Lithium hydroxide (0.03 g, 1.11 mmol) was added to a biphasic solution of the methyl ester **4.13** (0.10 g, 0.028 mmol) in THF (0.6 mL) and water (0.6 mL), and the reaction mixture was stirred at room temperature for 4 hours. The mixture was acidified with pH 2 buffer, and concentrated *in vacuo*. The residue was partitioned between pH 2 buffer and EtOAc, and the separated aqueous phase was further extracted with EtOAc (3 x 10 mL). The combined organic phases were dried over Na_2SO_4, and concentrated *in vacuo* to give the acid **4.14** as a bright white solid (0.09 g, 0.026 mmol, 93 %); $[\alpha]_D^{24}$ -64.7 (c = 0.61, $CHCl_3$); m.p. 138 – 140 °C; υ_{max}/cm^{-1} 3274 (N-H), 1711 (acid C=O), 1693 (carbamate C=O), 1646 (amide C=O), 1533 (N-H); δ_H (400 MHz, $CDCl_3$) 12.32 (1H, br. s., CO_2H), 7.48 (1H, d, *J* 6.5 Hz, N*H*CH), 7.23 - 7.10 (5H, m, 5 x Ar C-*H*), 4.97 (2H, s, CH_2Ph), 4.87 (1H, dd, *J* 10.0, 6.5 Hz, NHC*H*), 2.39 (3H, s, oxazole CH_3), 2.01 - 1.89 (1H, m, C*H*CH_3), 1.46 (1H, dqd, *J* 15.0, 7.0, 4.0 Hz, CH_2CH_3), 1.27 - 1.14 (1H, m, CH_2CH_3), 0.85 - 0.79 (6H, m, CHCH_3, CH_2CH_3); δ_C (100 MHz, $CDCl_3$) 164.4, 163.9, 157.2, 156.8 (*C*O_2H, *C*$O_2{}^t$Bu, 2 x oxazole *C*), 136.3 (*ipso C*), 128.5, 128.3, 128.0 (*ortho*, *meta*, *para C*-H), 127.0 (oxazole *C*), 67.0 (*C*H_2Ph), 54.0 (NH*C*H), 39.1 (*C*HCH_3), 25.1 (*C*H_2CH_3), 15.5 (CH*C*H_3), 11.7, 11.2 (CH_2*C*H_3, oxazole *C*H_3); *m/z* (ESI+) 347.1 ([M+H]+); HR-ESIMS: calculated for $C_{18}H_{23}N_2O_5$: 347.1601, found 347.1594 $[M+H]^+$.

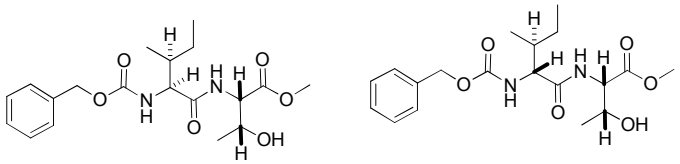
(2*S*, 3*S*) and (2*R*, 3*S*) *N*-carboxybenzyl-isoleucine 4.15



A solution of benzyl chloroformate (1.3 mL, 9.2 mmol) in THF (10 mL) was added dropwise to a stirred and cooled solution of L and D-*allo*-isoleucine **3.12** (1.0 g, 7.6 mmol) in aqueous NaOH solution (1M, 17 mL, 17.0 mmol) at 0 °C. Following addition, the reaction mixture was allowed to reach room temperature overnight. Sufficient aqueous NaOH solution (1M) was added until the mixture was above pH8 and the mixture extracted with diethyl ether (2 x 10 mL). The aqueous phase was acidified to pH 2 with aqueous hydrochloric acid solution (1M) and extracted with EtOAc (3 x 10 mL). The combined organic extracts were washed with saturated aqueous NaCl solution (10 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford the protected amino-acid **4.15** as a sticky pale yellow oil (1.95 g, 7.40 mmol, 97 %, 83: 17 mix of (2*S*, 3*S*) and (2*R*, 3*S*), calculated by ^{1}H NMR); υ_{max}/cm^{-1} 3308 (N-H), 2965 (O-H), 1701 (C=O), 1517 (N-H); δ_H (400 MHz, $CDCl_3$) 8.75 ((2*S,* 3*S*), br. s., 1H, CO_2H; (2*R,* 3*S*), br. s., 1H, CO_2H), 7.44 - 7.28 ((2*S,* 3*S*), m, 5H, Ar C-*H*; (2*R,* 3*S*), m, 5H, Ar C-*H*), 5.39 ((2*S,* 3*S*), d, *J* 9.0 Hz, N*H*CH), 5.32 ((2*R,* 3*S*), d, *J* 9.0 Hz, N*H*CH) 5.18 - 5.05 ((2*S,* 3*S*), 2H, m, CH_2Ph; (2*R,* 3*S*), 2H, m, CH_2Ph), 4.52 ((2*R,* 3*S*), 1H, dd, *J* 9.5, 4.0 Hz, NHC*H*), 4.40 ((2*S,* 3*S*), 1H, dd, *J* 9.0, 4.5 Hz, NHC*H*), 2.08 - 1.79 ((2*S,* 3*S*), 1H, m, C*H*CH_3; (2*R,* 3*S*), 1H, m, C*H*CH_3), 1.59 - 1.34 ((2*S,* 3*S*), 1H, m, CH_2CH_3; (2*R,* 3*S*), 1H, m, CH_2CH_3), 1.34 - 1.14 ((2*S,* 3*S*), 1H, m, CH_2CH_3; (2*R,* 3*S*), 1H, m, CH_2CH_3), 1.04 - 0.84 ((2*S,* 3*S*), 6H, m, CH_2CH_3, $CHCH_3$; (2*R,* 3*S*), 6H, m, CH_2CH_3, $CHCH_3$); δ_C (100 MHz, $CDCl_3$) 177.2, 176.7 ((2*S,* 3*S*), *C*O_2H; (2*R,* 3*S*), *C*O_2H), 156.5, 156.3 ((2*S,* 3*S*), *C*O_2Bn; (2*R,* 3*S*), *C*O_2Bn), 136.1 ((2*S,* 3*S*), *ipso C*; (2*R,* 3*S*), *ipso C*), 128.5, 128.2, 128.1 ((2*S,* 3*S*), *ortho, meta* and *para C*-H; (2*R,* 3*S*), *ortho, meta* and *para C*-H), 67.6, 67.1 ((2*S,* 3*S*), *C*H_2Ph; (2*R,* 3*S*), *C*H_2Ph), 58.8, 58.2 ((2*S,* 3*S*), NH*C*H; (2*R,* 3*S*), NH*C*H), 37.7, 37.4 ((2*S,* 3*S*), *C*HCH_3; (2*R,* 3*S*), *C*HCH_3), 26.2, 24.8 ((2*S,* 3*S*), *C*H_2CH_3; (2*R,* 3*S*), *C*H_2CH_3), 15.4,

14.3 ((2*S,* 3*S*), CH*C*H$_3$; (2*R,* 3*S*), CH*C*H$_3$), 11.6 ((2*S,* 3*S*), *C*H$_2$CH$_3$; (2*R,* 3*S*), *C*H$_2$CH$_3$); *m/z* (ESI+) 288.1 ([M+Na]+); HR-ESIMS: calculated for $C_{14}H_{19}NO_4Na$: 288.1206, found 288.1195 $[M+Na]^+$.

(2*S*, 3*S*) and (2*R*, 3*S*) Dipeptide 4.16

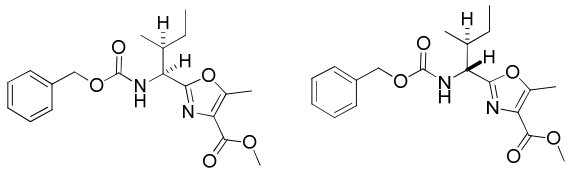


Acetyl chloride (21.0 mL, 298 mmol) was added dropwise to MeOH (130 mL) at 0°C. To this solution was added L-threonine (5.90 g, 49.7 mmol) and the solution was heated at reflux for 18 hours. The reaction mixture was then concentrated *in vacuo* to give L-threonine methyl ester as the hydrochloride salt which was used without further purification.

1-Hydroxybenzotriazole (1.10 g, 7.20 mmol) was added to a solution of *N*-carboxybenzyl-isoleucine as a mix of L-isoleucine and D-*allo*-isoleucine diastereomers **4.15** (12.8 g, 48.3mmol) in EtOH (200 mL) and the mixture stirred at room temperature for 15 minutes. To this was added a solution of the crude threonine methyl ester hydrochloride (49.7 mmol) in EtOH (50 mL) and the reaction mixture cooled to 0°C. *N*-Methylmorpholine (16.8 mL, 155 mmol) was added and the reaction mixture stirred at 0-5°C for 15 minutes. EDCI (11.1 g, 57.9 mmol) was added and the reaction mixture stirred to room temperature over 18 hours. The resulting orange solution was acidified using pH 2 buffer and concentrated *in vacuo*. EtOAc (200 mL) was added and the phases separated. The organic phases was washed with pH 2 buffer (100 mL), water (100 mL), saturated aqueous $NaHCO_3$ solution (100 mL) and saturated aqueous NaCl solution (100 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the dipeptide **4.16** as a bright white solid (15.0 g, 39.4 mmol, 82 %, 83:17 mix of (2*S*, 3*S*) and (2*R*, 3*S*), calculated by ^{1}H NMR); υ_{max}/cm^{-1} 3307 (N-H), 3185 (O-H), 1729 (ester C=O), 1688 (carbamate

C=O), 1647 (amide C=O), 1534 (N-H); δ_H (400 MHz, DMSO-d_6) 8.00 - 7.58 ((2*S,* 3*S*), 1H, m, CON*H*CHCO$_2$Me; (2*R,* 3*S*), 1H, m, CON*H*CHCO$_2$Me), 7.49 - 7.21 ((2*S,* 3*S*), 6H, m, CO$_2$N*H*CH, 5 x Ar C-*H*; (2*R,* 3*S*), 6H, m, CO$_2$N*H*CH, 5 x Ar C-*H*), 5.09 - 5.00 ((2*S,* 3*S*), 2H, m, C*H*$_2$Ph; (2*R,* 3*S*), 2H, m, C*H*$_2$Ph), 4.98 ((2*S,* 3*S*), 1H, d, *J* 5.5 Hz, CHO*H*; (2*R,* 3*S*), 1H, d, *J* 5.5 Hz, CHO*H*), 4.29 ((2*S,* 3*S*), 1H, dd, *J* 8.5, 3.5 Hz, CONHC*H*CO$_2$Me; (2*R,* 3*S*), 1H, dd, *J* 8.5, 3.5 Hz, CONHC*H*CO$_2$Me), 4.21 - 4.07 ((2*S,* 3*S*), 1H, m, C*H*OH; (2*R,* 3*S*), 2H, m, C*H*OH, CO$_2$NHC*H*), 4.04 ((2*S,* 3*S*), 1H, dd, *J* 9.0, 8.0 Hz, CO$_2$NHC*H*), 3.71 - 3.50 ((2*S,* 3*S*), 3H, m, OC*H*$_3$; (2*R,* 3*S*), 3H, m, OC*H*$_3$), 1.93 - 1.59 ((2*S,* 3*S*), 1H, m, C*H*CH$_2$; (2*R,* 3*S*), 1H, m, C*H*CH$_2$), 1.59 - 1.26 ((2*S,* 3*S*), 1H, m, C*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, C*H*$_2$CH$_3$), 1.26 - 0.95 ((2*S,* 3*S*), 4H, m, CH(OH)C*H*$_3$, C*H*$_2$CH$_3$; (2*R,* 3*S*), 4H, m, CH(OH)C*H*$_3$, C*H*$_2$CH$_3$), 0.95 - 0.54 ((2*S,* 3*S*), 6H, m, CH$_2$C*H*$_3$, CH(CH$_2$)C*H*$_3$; (2*R,* 3*S*), 4H, m, CH$_2$C*H*$_3$, CH(CH$_2$)C*H*$_3$); δ_C (100 MHz, *DMSO-d$_6$*) 171.8, 171.0 ((2*S,* 3*S*), *C*ONH, *C*O$_2$CH$_3$; (2*R,* 3*S*), *C*ONH, *C*O$_2$CH$_3$), 156.0 ((2*S,* 3*S*), PhCH$_2$O*C*; (2*R,* 3*S*), PhCH$_2$O*C*), 137.1 ((2*S,* 3*S*), *ipso C*; (2*R,* 3*S*), *ipso C*), 128.3 ((2*S,* 3*S*), Ar *C*-H; (2*R,* 3*S*), Ar *C*-H), 127.7 ((2*S,* 3*S*), *para C*-H; (2*R,* 3*S*), *para C*-H),127.6 ((2*S,* 3*S*), Ar *C*-H; (2*R,* 3*S*), Ar *C*-H), 66.2 ((2*S,* 3*S*), *C*HOH; (2*R,* 3*S*), *C*HOH), 65.5, 65.3 ((2*S,* 3*S*), *C*H$_2$Ph; (2*R,* 3*S*), *C*H$_2$Ph), 59.1, 58.6 ((2*S,* 3*S*), CO$_2$NH*C*H; (2*R,* 3*S*), CO$_2$NH*C*H), 57.7, 57.6 ((2*S,* 3*S*), CONH*C*H; (2*R,* 3*S*), CONH*C*H), 51.8, 51.7 ((2*S,* 3*S*), O*C*H$_3$; (2*R,* 3*S*), O*C*H$_3$), 36.7, 36.3 ((2*S,* 3*S*), *C*HCH$_2$; (2*R,* 3*S*), *C*HCH$_2$), 24.8, 24.3 ((2*S,* 3*S*), *C*H$_2$CH$_3$; (2*R,* 3*S*), *C*H$_2$CH$_3$), 20.0 ((2*S,* 3*S*), CH(OH)*C*H$_3$; (2*R,* 3*S*), CH(OH)*C*H$_3$), 15.2 ((2*S,* 3*S*), CH(CH$_2$)*C*H$_3$; (2*R,* 3*S*), CH(CH$_2$)*C*H$_3$), 11.4, 10.9 ((2*S,* 3*S*), CH$_2$*C*H$_3$; (2*R,* 3*S*), CH$_2$*C*H$_3$); *m/z* (ESI+) 403.1 ([M+Na]+, 100.0%); HR-ESIMS: calculated for $C_{19}H_{29}N_2O_6$: 381.2020, found 381.2019 $[M+H]^+$.

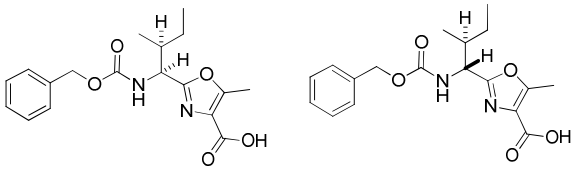
(2*S*, 3*S*) and (2*R*, 3*S*) Oxazole Methyl Ester 4.17



Prepared as for the single diastereomer, using a mix of L-isoleucine and D-*allo*-isoleucine dipeptide **4.16** (2.0 g, 5.55 mmol), SO_3.pyridine (0.96 g, 6.04 mmol), DIPEA (1.3 mL, 7.89 mmol), dry DMSO (2 mL) and dry CH_2Cl_2 (15 mL) for the oxidation and PPh_3 (0.78 g, 2.94 mmol), iodine (0.68 g, 2.70 mmol), triethylamine (0.94 mL, 6.74 mmol) and THF (16 mL) for the cyclodehydration to give the oxazole methyl ester **4.17** as a bright white solid (0.33 g, 0.92 mmol, 17 % over 2 steps, 83: 17 mix of (2*S*, 3*S*) and (2*R*, 3*S*), calculated by ^{1}H NMR); υ_{max}/cm^{-1} 3309 (N-H), 1717 (ester C=O), 1679 (carbamate C=O), 1615 (amide C=O), 1532 (N-H); δ_H (400 MHz, $CDCl_3$) 7.36 - 7.13 ((2*S,* 3*S*), 5H, m, Ar C-*H*; (2*R,* 3*S*), 5H, m, Ar C-*H*), 5.53 – 5.44 ((2*S,* 3*S*), 1H, m, N*H*CH; (2*R,* 3*S*), 1H, m, N*H*CH), 5.09 - 4.98 ((2*S,* 3*S*), 1H, m, CH_2Ph; (2*R,* 3*S*), 1H, m, CH_2Ph), 4.89 ((2*S,* 3*S*), 1H, dd, *J* 9.5, 5.0 Hz, NHC*H*), 4.79 ((2*R,* 3*S*), 1H, dd, *J* 9.0, 6.5 Hz, NHC*H*), 3.83 ((2*S,* 3*S*), 3H, s, OCH_3; (2*R,* 3*S*), 3H, s, OCH_3), 2.53 ((2*S,* 3*S*), 3H, s, oxazole CH_3; (2*R,* 3*S*), 3H, s, oxazole CH_3), 1.96 – 1.81 ((2*S,* 3*S*), 1H, m, C*H*CH$_3$; (2*R,* 3*S*), 1H, m, C*H*CH$_3$) 1.51 - 1.25 ((2*S,* 3*S*), 1H, m, CH_2CH$_3$; (2*R,* 3*S*), 1H, m, CH_2CH$_3$), 1.24 - 1.01 ((2*S,* 3*S*), 1H, m, CH_2CH$_3$; (2*R,* 3*S*), 1H, m, CH_2CH$_3$), 0.95 - 0.71 ((2*S,* 3*S*), 6H, m, CHCH_3, CH$_2$CH_3; (2*R,* 3*S*), 6H, m, CHCH_3, CH_2CH$_3$); δ_C (100 MHz, $CDCl_3$) 162.6 ((2*S,* 3*S*), *C*O$_2$Me; (2*R,* 3*S*), *C*O$_2$Me), 161.8, 161.6 ((2*S,* 3*S*), *C*O$_2$Bn; (2*R,* 3*S*), *C*O$_2$Bn), 156.2, 155.8 ((2*S,* 3*S*), oxazole *C*; (2*R,* 3*S*), oxazole *C*), 136.2 ((2*S,* 3*S*), *ipso C*; (2*R,* 3*S*), *ipso C*), 128.4, 128.0, 127.9 ((2*S,* 3*S*), *ortho, meta* and *para C*-H; (2*S,* 3*S*), *ortho, meta* and *para C*-H), 127.3 ((2*S,* 3*S*), oxazole *C*; (2*R,* 3*S*), oxazole *C*), 66.9 ((2*S,* 3*S*), Ph*C*H$_2$; (2*R,* 3*S*), Ph*C*H$_2$), 53.7, 53.0 ((2*S,* 3*S*), NH*C*H; (2*R,* 3*S*), NH*C*H), 51.8 ((2*S,* 3*S*), O*C*H$_3$; (2*R,* 3*S*), O*C*H$_3$), 39.1, 39.0 ((2*S,* 3*S*), *C*HCH$_3$; (2*R,* 3*S*), *C*HCH$_3$), 25.8, 24.9 ((2*S,* 3*S*), *C*H$_2$CH$_3$; (2*R,* 3*S*), *C*H$_2$CH$_3$), 15.1, 14.5 ((2*S,* 3*S*), CH*C*H$_3$; (2*R,* 3*S*),

CH*C*H$_3$), 12.1, 12.0 ((2*S,* 3*S*), oxazole *C*H$_3$; (2*R,* 3*S*), oxazole *C*H$_3$), 11.4, 11.1 ((2*S,* 3*S*), CH$_2$*C*H$_3$; (2*R,* 3*S*), CH$_2$*C*H$_3$); *m/z* (ESI+) 383.1 ([M+Na]+, 100.0%), 361.2 ([M+H]+, 8.0%); HR-ESIMS: calculated for $C_{19}H_{25}N_2O_5$: 361.1758, found 361.1754 $[M+H]^+$.

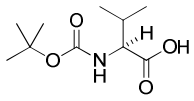
(2*S*, 3*S*) and (2*R*, 3*S*) Oxazole Acid 4.18



Prepared as for the single diastereomer, using a mix of L-isoleucine and D-*allo*-isoleucine diastereomers **4.17** (0.10 g, 0.28 mmol), lithium hydroxide (0.03 g, 1.11 mmol), THF (0.6 mL) and water (0.6 mL) to give the acid **4.18** as a white solid (0.08 g, 0.23 mmol, 82 %, 84: 16 mix of (2*S*, 3*S*) and (2*R*, 3*S*), calculated by ^{1}H NMR); υ_{max}/cm^{-1} 3326 (N-H), 1710 (acid C=O), 1620 (amide C=O), 1533 (N-H); δ_H (400 MHz, $CDCl_3$) 7.71 - 7.39 ((2*S,* 3*S*), 1H, m, N*H*CH; (2*R,* 3*S*), 1H, m, N*H*CH), 7.46 - 7.03 ((2*S,* 3*S*), 5H, m, 5 x Ar C-*H*; (2*R,* 3*S*), 5H, m, 5 x Ar C-*H*), 5.15 - 4.99 ((2*S,* 3*S*), 2H, m, C*H*$_2$Ph; (2*R,* 3*S*), 3H, m, C*H*$_2$Ph, NHC*H*), 4.94 ((2*S,* 3*S* , 1H, dd, *J* 10.0, 7.0 Hz, NHC*H*), 2.54 ((2*R,* 3*S*), 3H, s, oxazole C*H*$_3$), 2.48 ((2*S,* 3*S*), 3H, s, oxazole C*H*$_3$), 2.06 - 1.93 ((2*S,* 3*S*), 1H, m, C*H*CH$_3$; (2*R,* 3*S*), 1H, m, C*H*CH$_3$), 1.63 - 1.38 ((2*S,* 3*S*), 1H, m, C*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, C*H*$_2$CH$_3$), 1.36 - 1.22 ((2*S,* 3*S*), 1H, m, C*H*$_2$CH$_3$; (2*R,* 3*S*), 1H, m, C*H*$_2$CH$_3$), 1.02 - 0.85 ((2*S,* 3*S*), 1H, m, C(C*H*$_3$)$_3$; (2*R,* 3*S*), 1H, m, C(C*H*$_3$)$_3$); δ_C (100 MHz, $CDCl_3$) 164.4, 164.1, 164.0, 157.4, 157.2, 156.9, 156.8 ((2*S,* 3*S*), *C*O$_2$H, *C*O$_2$^{t}Bu, 2 x oxazole *C*; (2*R,* 3*S*), *C*O$_2$H, *C*O$_2$^{t}Bu, 2 x oxazole *C*); 136.5, 136.3 ((2*S,* 3*S*), *ipso C*; (2*R,* 3*S*), *ipso C*), 128.3, 128.0, 127.9, 127.8 ((2*S,* 3*S*), *ortho, meta* and *para C*-H; (2*R,* 3*S*), *ortho, meta* and *para C*-H), 126.9 ((2*S,* 3*S* oxazole *C*; (2*R,* 3*S*), oxazole *C*), 67.0, 66.9 ((2*S,* 3*S*), *C*H$_2$Ph; (2*R,* 3*S*), *C*H$_2$Ph), 54.0, 53.4 ((2*S,* 3*S*), NH*C*H; (2*R,* 3*S*), NH*C*H), 39.1, 39.0 ((2*S,* 3*S*), *C*HCH$_3$; (2*R,* 3*S*), *C*HCH$_3$), 26.0, 25.0 ((2*S,* 3*S*), *C*H$_2$CH$_3$; (2*R,* 3*S*), *C*H$_2$CH$_3$), 15.5, 14.2 ((2*S,* 3*S*), CH*C*H$_3$; (2*R,* 3*S*), CH*C*H$_3$), 11.8, 11.7, 11.5, 11.2 ((2*S,* 3*S*), oxazole *C*H$_3$, CH$_2$*C*H$_3$;

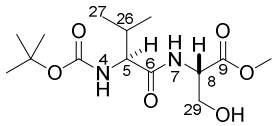
(2*R,* 3*S*), oxazole *C*H_3, CH_2*C*H_3); *m/z* (ESI-) 345.1 ([M-H], 100.0%); HR-ESIMS: calculated for $C_{18}H_{22}N_2O_5Na$: 369.1421, found 369.1425 $[M+Na]^+$.

***N-tert*-Butyloxycarbonyl-L-valine 4.19**



Method modified from literature procedure by Garner *et al.*[11] A solution of di-tert-butyl dicarbonate (112 g, 512 mmol) in dioxane (300 mL) was added dropwise to a stirred and cooled solution of L-valine (50.0 g, 427 mmol) in aqueous NaOH solution (1 M, 875 mL) at 0 °C. The pH of the solution was adjusted to 9 using aqueous NaOH solution and the reaction mixture was allowed to reach room temperature overnight. The pH was adjusted to between 9 and 10, and the reaction mixture extracted with diethyl ether (3 x 250 mL). The aqueous phase was acidified to pH 2 with concentrated H_2SO_4 and extracted with EtOAc (5 x 100 mL). The combined organic extracts were washed with saturated aqueous NaCl solution (100 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the protected amino-acid as bright white crystals (87.5 g, 403 mmol, 94 %); m.p. 76 - 78 °C, (lit.[12] 77 - 78 °C); $[\alpha]_D^{25}$ +6.6 (c = 1.1, $CHCl_3$), (lit. [13] $[\alpha]_D^{23}$ +11.7 (c = 2.35, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3320 (NH), 2967 (OH), 1711 (acid C=O), 1640 (amide C=O), 1509 (NH); δ_H (400 MHz, $CDCl_3$) 9.15 (1H, br.s., major and minor rotamer CO_2*H*), 6.21 (1H, d, *J* 6.5 Hz, minor rotamer N*H*CH), 5.08 (1H, d, *J* 9.0 Hz, major rotamer N*H*CH), 4.25 (1H, dd, *J* 9.0, 4.5 Hz, major rotamer NHC*H*), 4.13 - 3.93 (1H, m, minor rotamer NHC*H*), 2.29 – 2.13 (1H, m, major and minor rotamer C*H*$(CH_3)_2$), 1.47-1.40 (9H, m, major and minor rotamer C(C$H_3)_3$), 0.99 (1H, d, *J* 7.0 Hz, major and minor rotamer CH(C$H_3)_2$), 0.93 (1H, d, *J* 7.0 Hz, major and minor rotamer CH(C$H_3)_2$); δ_C (100 MHz, $CDCl_3$) 176.8 (*C*O_2H), 155.8 (*C*ONH), 80.0 (*C*$(CH_3)_3$), 58.4 (*C*HNH), 30.9 (*C*H$(CH_3)_2$), 28.3 (C(*C*$H_3)_3$), 19.0, 17.4 (CH(*C*$H_3)_2$); *m/z* (ESI+) 240.1 ([M+Na]+, 100.0%); HR-ESIMS: calculated for $C_{10}H_{19}NO_4Na$: 240.1206, found 240.1208 $[M+Na]^+$.The data are consistent with that previously reported.[14]

***N-tert*-Butyloxycarbonyl-L-valyl-L-serine methyl ester 4.20**

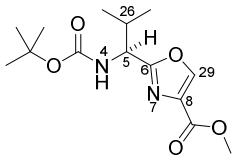


Acetyl chloride (40.6 mL, 571 mmol) was added dropwise with stirring to MeOH (240 mL) at 0 °C. The mixture was allowed to reach room temperature and L-serine (10.0 g, 95.2 mmol) was added and the mixture was heated to reflux for 7 hours. The reaction mixture was concentrated *in vacuo* to give the crude methyl ester as the hydrochloride salt, which was used without further purification assuming quantitative yield.

A solution of *N-tert*-butyl carbonyl-L-valine **4.19** (20.0 g, 95.2 mmol) and HOBt (88 %, 2.20 g, 14.3 mmol) in EtOH (150 mL) was stirred at room temperature for 15 minutes, then cooled to 0 - 5°C. To this was added a solution of the crude serine methyl ester (95.2 mmol) and *N*-methyl morpholine (33.1 mL, 305 mmol) in EtOH (250 mL) and the resulting colourless solution stirred at 0 - 5°C for 15 minutes. EDCI (21.8g, 114mmol) was added and the reaction mixture was allowed to reach room temperature overnight to give a pale yellow solution. The reaction was acidified using pH 2 buffer and *N*-methyl morpholine hydrosulfate removed by filtration. The filtrate was concentrated *in vacuo*, and extracted with EtOAc (4 x 100 mL). The combined organic extracts were washed with pH 2 buffer (100 mL), water (100 mL), saturated aqueous $NaHCO_3$ solution (100 mL) and saturated aqueous NaCl solution (100mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the dipeptide as a white powdery solid (19.2 g, 60.3 mmol, 63 %. The reaction was also performed with 10.0 g of compound **4.20** with a yield of 94 %); m.p. 77 - 78 °C (lit.[15] 77.5 – 79.5 °C); $[\alpha]_D^{26}$ -22.7 (c = 1.00, MeOD), (lit.[15] $[\alpha]_D^{20}$ = -21.1 (c = 1.03, MeOH)); υ_{max}/cm^{-1} (neat) 3318 (NH), 2963 (OH), 1747 (ester C=O), 1648 (amide C=O), 1519 (NH); δ_H (400 MHz, $CDCl_3$) 7.41 (1H, d, *J* 7.5 Hz, N7-*H*), 5.56 (1H, d, *J* 8.5 Hz, N4-*H*), 4.61 (1H, td, *J* 8.0, 4.0 Hz, C8-*H*), 4.11 (1H, t, *J* 6.0 Hz, CH_2OH), 3.97 (1H, t, *J* 8.0 Hz, C5-*H*), 3.93 - 3.75 (2H, m, CH_2OH), 3.68 (3H, s, OCH_3), 2.00

(1H, m, C26-*H*), 1.40 - 1.31 (9H, m, C(CH_3)$_3$), 0.92 (3H, d, *J* 7.0 Hz, C26(CH_3)$_2$), 0.88 (d, *J* 7.0 Hz, C26(CH_3)$_2$); ^{13}C (100 MHz, $CDCl_3$) 172.2 (*C*O), 170.7 (*C*O), 156.1 (*C*O$_2^t$Bu), 79.7 (*C*(CH$_3$)$_3$), 62.3 (C8-*C*H$_2$), 59.7 (*C*26), 54.4 (*C8*-H), 52.4 (O*C*H$_3$), 31.0 (*C*26), 28.1 (C(*C*H$_3$)$_3$), 18.9, 17.8 (C26(*C*H$_3$)$_2$); *m/z* (ESI+) 341.2 ([M+Na]+ 100%); HR-ESIMS: calculated for $C_{14}H_{26}N_2O_6Na$: 341.1683, found 341.1676 [M+Na]$^+$. The data are consistent with that previously reported.[13]

2-[(*S*)-*N*-*tert*-Butyloxycarbonylamino-2-methylpropyl]-4-carbomethoxyoxazole 4.21



Method modified from literature procedure of Zylstra *et al.*[16] Triphenylphosphine (22.2 g, 84.8 mmol) was added to a stirred solution of dipeptide **4.20** (15.0 g, 47.1 mmol) in dry CH_2Cl_2 (150 mL) and acetonitrile (35 mL) under nitrogen. To this solution was added diisopropylethylamine (14.0 mL, 84.8 mmol) and the reaction mixture was cooled to 0 -5 °C. After 20 minutes carbon tetrachloride (8.2 mL, 84.8 mmol) was added dropwise. The reaction was stirred at 0-5 °C for a further 15 minutes and then allowed to reach room temperature overnight. Water (250 mL) was added, and the mixture extracted with CH_2Cl_2 (2 x 50 mL). The combined organic phases were concentrated *in vacuo* and the residue partitioned between EtOAc (200 mL) and water (100 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ solution (100 mL) and saturated aqueous NaCl solution (100 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give a brown solid. This was purified by silica chromatography (50 % EtOAc: pet. ether) to give the oxazoline as a yellow oil (4.82 g, 16.1 mmol) which was used immediately.

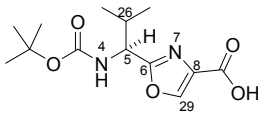
A solution of oxazoline (4.82 g, 16.1 mmol) in dry CH_2Cl_2 (100 mL) was cooled to 0 °C under nitrogen. 1, 8-Diazabicyclo[5.4.0]undec-7-ene (7.4 mL, 49.7 mmol) was added and the mixture stirred at 0 °C for 10 minutes. Bromotrichloromethane (4.9

mL, 49.7 mmol) was added dropwise and the reaction stirred at room temperature for 6 hours. Aqueous citric acid solution (10 % w/w, 250 mL) was added and the phases separated. The aqueous phase was extracted with CH_2Cl_2 (2 x 100 mL) and the combined organic phases dried over Na_2SO_4 and concentrated *in vacuo*. The oxazole **4.21** was obtained by silica chromatography (50 % EtOAc: pet. ether) as a bright white solid (3.9 g, 13.1 mmol, 28 % over 2 steps. The reaction was also performed with 10.0 g of compound **4.20** with a yield of 35 % and with 3.0 g of compound **4.20** with a yield of 46 %); m.p. 127 - 129 °C (lit.[17] 120 - 124°C); $[\alpha]_D^{23}$ -37.7 (c = 0.60, $CHCl_3$), (lit.[18] $[\alpha]_D^{25}$ -36.4 (c = 0.5, $CHCl_3$)); υ_{max}/cm^{-1} (neat) 3288 (NH), 2967 (aromatic C-H), 1749 (ester C=O), 1698 (amide C=O), 1538 (NH); δ_H (400 MHz, $CDCl_3$) 8.19 (1H, s, C29-*H*), 5.29 (1H, d, *J* 9.5 Hz, N4-*H*), 4.81 (1H, dd, *J* 9.0, 6.5 Hz, C5-*H*), 3.92 (3H, s, OCH_3), 2.20 (1H, octet, *J* 6.5 Hz, C26-*H*), 1.44 (9H, s, $C(CH_3)_3$), 0.93 (6H, 2 x d, *J* 8.0 Hz, $C26(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 165.2 (*C6*), 161.6 (CO_2Me), 155.3 (*C*ONH), 143.8 (*C29*-H), 133.1 (*C8*), 80.0 ($C(CH_3)_3$), 54.1 (*C5*-H), 52.2 (CO_2CH_3), 32.9 (*C26*), 28.2 ($C(CH_3)_3$), 18.7, 17.8 ($C26(CH_3)_2$); *m/z* (ESI+) 321.1 ([M+Na]+ 100%); HR-ESIMS: calculated for $C_{14}H_{22}N_2O_5Na$: 321.1421, found 321.1418 $[M+Na]^+$. The data are consistent with that previously reported.[19]

Oxazole **4.21** was also prepared using (diethylamino)sulphur trifluoride. Method modified from literature procedure of Pattenden *et al.*[4] A solution of dipeptide **4.20** (7.0 g, 22.0 mmol) in dry CH_2Cl_2 (150 mL) under nitrogen was cooled to -78 °C and (diethylamino)sulfur trifluoride (4.1 mL, 31 mmol) was added dropwise and the reaction mixture stirred at -78 °C for 1.5 hrs. The mixture was allowed to reach room temperature over 15 minutes before carefully quenching with saturated aqueous $NaHCO_3$ solution. The mixture was stirred for 15 minutes and the phases were separated. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to give the crude oxazoline as a clear yellow oil. This was dissolved in dry CH_2Cl_2 (150 mL) under a nitrogen atmosphere and cooled to 0 °C. $BrCCl_3$ (2.7 mL, 27.0 mmol)

was added dropwise and the reaction mixture was stirred for 5 minutes. 1, 8-Diazabicyclo[5.4.0]undec-7-ene (3.8 mmol, 25.3 mmol) was added and the reaction mixture was stirred to room temperature overnight. The reaction was quenched with 10% aqueous citric acid solution (100 mL) and the phases were separated. The aqueous phase was further extracted with CH_2Cl_2 (2 x 50 mL) and the organic phases were combined, washed with saturated NaCl solution (10 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give a beige solid. The oxazole **4.21** was obtained by silica chromatography (50 % EtOAc: pet. ether) as a pale yellow solid (3.03 g, 10.2 mmol, 46 %. The reaction was also performed with 7.0 g of compound **4.20** with a yield of 47 % and with 7.0 g of compound **4.20** with a yield of 39 %). The characterization data was identical to that prepared in the previous method.

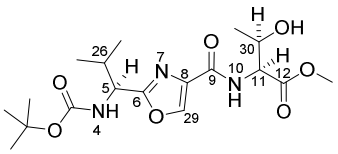
Oxazole Acid 4.22



LiOH (0.16 g, 6.70 mmol) and water (3 mL) were added to a solution of methyl ester **4.21** (0.50 g, 1.68 mmol) in THF (3 mL). The biphasic mixture was stirred at room temperature for 4 hours and acidified with pH 2 buffer. The mixture was concentrated *in vacuo*, EtOAc (10 mL) was added and the phases were separated. The aqueous phase was extracted with EtOAc (3 x 3mL) and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give acid **4.22** as a bright white solid (0.45 g, 1.58 mmol, 94 %); m.p. 146 - 146 °C, (lit.[17] m.p. 154 - 155 °C); $[\alpha]_D^{23}$ -76.3 (c = 0.65, $CHCl_3$), (lit.[17] $[\alpha]_D^{23}$ -28.9 (c = 1.5, $CHCl_3$)); υ_{max}/cm^{-1} (neat) 3321 (NH), 3074 (OH), 2971 (aromatic C-H), 1737 (acid C=O), 1514 (NH); δ_H (400 MHz, $CDCl_3$) 10.95 (1H, br.s., CO_2H), 8.30 (1H, s, C29-*H*), 6.40 (1H, d, *J* 9.0 Hz, N4-*H*), 4.84 (1H, dd, *J* 8.5, 7.0 Hz, C5-*H*), 2.39 - 2.16 (1H, m, C26-*H*), 1.40 (9H, s, $C(CH_3)_3$), 0.98 (3H, d, *J* 6.5 Hz, $C26(CH_3)_2$), 0.91 (3H, d, *J* 6.5 Hz, $C26(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 166.7 (*C6*), 163.6 (CO_2H), 155.9 (*C*ONH), 144.6 (*C29*-H), 133.2 (*C8*), 79.9 ($C(CH_3)_3$), 54.6 (*C5*), 32.8 (*C*26), 28.2 ($C(CH_3)_3$), 18.9,

18.2 (*C*26(*C*H$_3$)$_2$); *m/z* (ESI-) 283.1 ([M-H] 100%); HR-ESIMS: calculated for $C_{13}H_{19}N_2O_5$: 283.1299, found 283.1302 [M-H]$^-$. The data are consistent with that previously reported.[18]

Oxazole Methyl Ester 4.23

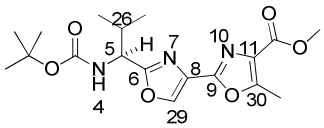


Threonine methyl ester hydrochloride was prepared as before, using L-threonine (0.20 g, 1.68 mmol) and was used without further purification and assuming quantitative yield.

A solution of the acid **4.22** (0.45 g, 1.58 mmol) and HOBt (88 %, 0.04 g, 0.25 mmol) in EtOH (3 mL) was stirred at room temperature for 5 minutes. To this was added a solution of L-threonine methyl ester hydrochloride (1.68 mmol) in EtOH (5 mL), and the mixture was cooled to 0 - 5 °C. *N*-Methyl morpholine (0.60 mL, 5.52 mmol) was added and the mixture stirred at 0 - 5 °C for 15 minutes. EDCI (0.39 g, 2.02 mmol) was added and the reaction allowed to reach room temperature overnight. The reaction was acidified with pH 2 buffer, and the inorganics removed by filtration. The filtrate was concentrated *in vacuo*, and EtOAc (10 mL) added. The phases were separated and the aqueous phase further extracted (2 x 5 mL). The combined organic extracts were washed with pH 2 buffer (10 mL), water (10 mL), saturated aqueous $NaHCO_3$ solution (10 mL) and saturated aqueous NaCl solution, dried over Na_2SO_4 and concentrated *in vacuo* to give the coupled product **4.23** as an off white solid (0.63 g, 1.58 mmol, 99 %. The reaction was also performed with 3.31 g of compound **4.22** with a yield of 90 %, with 3.25 g of compound **4.22** with a yield of 88 %, with 2.37 g of compound **4.22** with a yield of 94 % and with 0.50 g of compound **4.22** with a yield of 95 %); m.p. 81 - 82 °C; $[\alpha]_D^{24}$ -30.1 (c = 1.01, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3368 (NH), 2975 (OH), 1718 (ester C=O), 1645 (amide C=O), 1511 (NH); δ_H (400 MHz, $CDCl_3$) 8.15 (1H, s, C29-*H*), 7.60 (1H, d, *J* 9.0 Hz,

N10-*H*), 5.25 (1H, d, *J* 9.0 Hz, N4-*H*), 4.78 (1H, dd, *J* 8.5, 6.0 Hz, C5-*H*), 4.74 (1H, dd, *J* 9.0, 2.5 Hz, C11-*H*), 4.49 – 4.41 (1H, m, C30-*H*), 3.79 (3H, s, OCH_3), 2.84 - 2.71 (1H, m, C30-O*H*), 2.12 (1H, octet, *J* 6.5 Hz, C26-*H*), 1.46 (9H, s, C(CH_3)$_3$), 1.28 (3H, d, *J* 6.5 Hz, C30-CH_3), 0.94 (d, *J* 7.0 Hz, C26(CH_3)$_2$), 0.92 (3H, d, *J* 6.5 Hz, C26(CH_3)$_2$); δ_C (100 MHz, $CDCl_3$) 171.1 (*C*O_2Me), 164.0 (*C6*), 161.0 (*C*O_2^{t}Bu), 155.4 (*C9*), 141.4 (*C29*-H), 135.5 (*C8*), 80.3 (*C*$(CH_3)_3$), 68.0 (*C30*), 57.1 (*C11*), 54.3 (*C5*), 52.6 (O*C*H_3), 32.7 (*C26*), 28.3 (C(*C*H_3)$_3$), 19.9 (C30-*C*H_3), 18.7, 18.0 (C26(*C*H_3)$_2$); *m/z* (ESI+) 422.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{18}H_{29}N_3O_7Na$: 422.1898, found 422.1902 $[M+Na]^+$.

Bisoxazole Methyl Ester 4.09

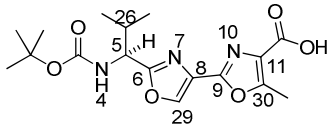


Method modified from literature procedure of Wipf *et al.*[6] A solution of the tripeptide **4.23** (3.37 g, 8.40 mmol) in dry CH_2Cl_2 (35.0 mL) was cooled to -20 °C under an atmosphere of nitrogen. Triethylamine (3.60 mL, 25.6 mmol) was added, followed by a suspension of SO_3.pyr (4.10 g, 25.6 mmol) in dry DMSO (15.0 mL). The mixture was stirred at -20 °C for 20 minutes and then at room temperature for 2.5 hours. The reaction was cooled to 0 °C and quenched with water (30 mL). The resulting mixture was stirred with an aqueous solution of oxone (0.5M, 145 mL) for 1 hour. The phases were separated, and the aqueous phase extracted with dichloromethane (2 x 10 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (5 mL) and saturated aqueous NaCl solution (25 mL), dried over Na_2SO_4 and concentrated *in vacuo***.** The ketone was obtained by silica chromatography (1:1 EtOAc: pet. ether) as an off-white solid (1.51 g, 3.98 mmol) which was used immediately.

The oxazole was prepared according to the method of Doi *et al.*[10] A solution of the ketone (0.22 g, 0.58 mmol), triphenylphosphine (0.29 g, 1.11 mmol) and CCl_4 (0.11

mL, 1.11 mmol) in dry CH_2Cl_2 (7.0 mL) was cooled to 0 °C under an atmosphere of nitrogen. To this was added diisopropylethylamine (0.37 mL, 2.21 mmol) and the reaction stirred at room temperature overnight. The solvent was evaporated and the bisoxazole **4.09** obtained by silica chromatography (1: 1 EtOAc: pet. ether) as a bright white crystalline solid (1.03 g, 2.71 mmol, 32% from alcohol **4.23**. The reaction was also performed with 3.83 g of compound **4.23** with a yield of 10 %); m.p. 128 - 130 °C; $[\alpha]_D^{24}$ -46.6 (c = 0.53, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3349 (NH), 2964 (aromatic C-H), 1712 (ester C=O), 1683 (amide C=O), 1519 (NH); δ_H (400 MHz, $CDCl_3$) 8.25 (1H, s, C29-*H*), 5.30 (1H, d, *J* 9.0 Hz, N4-*H*), 4.82 (1H, dd, *J* 9.0, 6.5 Hz, C5-*H*), 3.93 (3H, s, OC*H*$_3$), 2.70 (3H, s, C30-C*H*$_3$), 2.22 (1H, m, C26-*H*), 1.46 - 1.39 (9H, m, C(C*H*$_3$)$_3$), 0.95 (3H, d, *J* 7.5 Hz, C26(C*H*$_3$)$_2$), 0.93 (3H, d, *J* 7.5 Hz, C26(C*H*$_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 165.4 (*C6*), 164.5 (*C*O_2Me), 156.5 (*C30*), 155.3 (*C*ONH), 153.0 (*C9*), 138.8 (*C29*-H), 129.8 (*C8*), 128.3 (*C11*), 80.0 (*C*$(CH_3)_3$), 54.3 (*C5*), 52.0 (O*C*H_3), 33.0 (*C26*), 28.3 (C(*C*H_3)$_3$), 18.7, 18.0 (C26(*C*H_3)$_2$), 12.0 (C30-*C*H_3); *m/z* (ESI+) 402.0 ([M+Na] 100%); HR-ESIMS: calculated for $C_{18}H_{25}N_3O_6Na$: 402.1636, found 402.1640 $[M+Na]^+$.

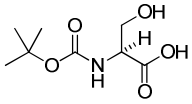
Bisoxazole Acid 4.24



Lithium hydroxide (0.13 g, 5.27 mmol) and water (3 mL) were added to a solution of the bioxazole methyl ester **4.09** (0.50 g, 1.32 mmol) in THF (3 mL) at room temperature. The reaction was stirred for 4 hours and then acidified using pH 2 buffer. The organic solvents were removed *in vacuo* and the residue was partitioned between EtOAc (15 mL) and pH 2 buffer. The separated aqueous phase was further extracted with EtOAc (3 x 5 mL) and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give the acid **4.24** as a white crystalline solid (0.46 g, 1.26 mmol, 95 %); m.p. 93 - 94 °C; $[\alpha]_D^{26}$ -45.0 (c = 1.05, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3295 (NH), 2117 (OH), 1696 (acid C=O), 1509 (NH); δ_H (400 MHz, $CDCl_3$)

8.29 (1H, s, C29-*H*), 7.68 (1H, br.s., major and minor rotamer, CO_2H), 6.38 (1H, d, *J* 6.0 Hz, minor rotamer N4-*H*), 5.46 (1H, d, *J* 9.5 Hz, major rotamer N4-*H*), 4.83 (1H, dd, *J* 9.5, 6.5 Hz, major rotamer C5-*H*), 4.71 - 4.59 (1H, m, minor rotamer C5-*H*), 2.72 (3H, s, major and minor rotamer, C30-CH_3), 2.21 (1H, oct., *J* 6.5 Hz, major and minor rotamer, C26-*H*), 1.48 - 1.37 (9H, m, major and minor rotamer, $C(CH_3)_3$), 0.96 (3H, d, *J* 7.0 Hz, major and minor rotamer, $C26(CH_3)_2$), 0.92 (3H, d, *J* 7.0 Hz, major and minor rotamer, $C26(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 165.6, 165.4 (*C6*, CO_2H), 157.3 (*C30*), 155.4 (*C*ONH), 153.0 (*C9*), 139.1 (*C29*-H), 129.5 (*C8*), 128.1 (*C9*), 80.1 ($C(CH_3)_3$), 54.3 (*C5*), 32.8 (*C26*), 28.2 ($C(CH_3)_3$), 18.7, 18.0. ($C26(CH_3)_2$), 12.2 (C30-CH_3): *m/z* (ESI+) 388.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{17}H_{23}N_3O_6Na$: 388.1479, found 388.1474 $[M+Na]^+$.

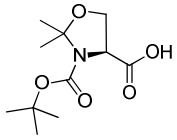
***N-tert*-Butoxy-L-serine 4.25**



A solution of di-tert-butyl dicarbonate (25.0 g, 114.5 mmol) in dioxane (75 mL) was added dropwise to a solution of L-serine (10.0 g, 95.2 mmol) in aqueous NaOH solution (1M, 195 mL, 195 mmol) at 0 °C. Following complete addition, the pH of the reaction mixture was adjusted to pH 9 - 10 and it was stirred at room temperature overnight to give a white suspension with a pH of 7 - 8. The pH was adjusted to 9 - 10 to give a clear, colourless solution. This was washed with Et_2O (2 x 50 mL), and the organic phases discarded. The aqueous phase was acidified to pH 2 buffer and extracted with EtOAc (4 x 100 mL). The organic extracts were combined, washed with saturated aqueous NaCl solution (100 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the protected amino acid **4.25** as a colourless oil (18.7 g, 91.1 mmol, 80%); $[\alpha]_D^{27}$ -4.5 (c = 1.3, H_2O), (lit.[20] $[\alpha]_D^{25}$ -7.6 (c = 2.6, H_2O)); υ_{max}/cm^{-1} (neat) 3333.7 (O-H), 1686 (C=O), 1512 (N-H); δ_H (400 MHz, $CDCl_3$) 6.67 (1H, br.s., major and minor rotamer, CO_2H), 5.89 (1H, d, *J* 7.0 Hz, major and minor rotamer,

N*H*), 4.40 - 4.30 (1H, m, NHC*H*, major rotamer), 4.28 - 4.17 (1H, m, NHC*H*, minor rotamer), 4.05 (1H, dd, *J* 11.0, 3.0 Hz, major and minor rotamer, CHCH_2), 3.85 (1H, dd, *J* 11.5, 3.0 Hz, major and minor rotamer, CHCH_2), 1.44 (9H, s, major and minor rotamer, C(CH_3)$_3$); δ_C (100 MHz, $CDCl_3$) 154.1 (*C*O_2H), 156.2 (*C*$O_2{}^t$Bu), 80.5 (*C*$(CH_3)_3$), 63.0 (NH*C*H), 55.6 (*C*H_2OH), 28.3 (C(*C*H_3)$_3$; *m/z* (ESI+) 228.0 ([M+Na] 100%); HR-ESIMS: calculated for $C_8H_{15}NO_5Na$: 228.0842, found 228.0841 $[M+Na]^+$. The data are consistent with that previously reported.[21]

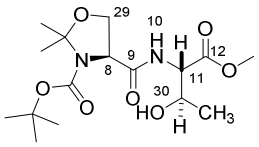
(*S*)-3-(*tert*-Butoxycarbonyl)-2,2-dimethyloxazolidine-4-carboxylic acid 4.26



Method modified from literature procedure of Trajkovic *et al* [22]. A solution of *N*-tert-butyloxycarbonyl-L-serine (13.3 g, 64.8 mmol), dimethoxypropane (64.0 g, 520 mmol) and toluene sulphonic acid monohydrate (1.11 g, 6.47 mmol) in CH_2Cl_2 (75 mL) was heated to reflux for 2 hours. After cooling to room temperature, the reaction mixture was concentrated *in vacuo* and the residue was partitioned between water (150 mL) and EtOAc (100 mL). The separated organic phase was washed with water (50 mL) and saturated aqueous NaCl solution (50 mL), dried over Na_2SO_4 and concentrated *in vacuo.* The protected amino-acid **4.25** was obtained, after drying under high vacuum, as a low melting point yellow solid (12.7 g, 51.8 mmol, 80%); m.p. <30°C; $[\alpha]_D^{25}$ -58.2 (c = 1.2, $CHCl_3$), (lit.[23] for opposite enantiomer $[\alpha]_D^{24}$ + 63.1 (c = 1.1, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3307 (O-H), 1687 (C=O), 1646 (C=O); δ_H (400 MHz, $CDCl_3$) 8.59 (1H, br. s., major and minor rotamer CO_2H), 4.49 (1H, dd, *J* 6.5, 2.0 Hz, minor rotamer C*H*CH_2), 4.37 (1H, dd, *J* 7.0 Hz, 3.0 Hz, major rotamer C*H*CH_2), 4.20 - 4.13 (1H, m, major and minor rotamer CHCH_2), 4.09 (1H, td, *J* 9.0, 2.5 Hz, CHCH_2), 1.64 (3H, s, major rotamer C(CH_3)$_2$), 1.59 (3H, s, 3H, s, minor rotamer C(CH_3)$_2$), 1.51 (3H, s, 3H, s, major rotamer C(CH_3)$_2$), 1.47 (12H, s, 3H, s, major rotamer C(CH_3)$_2$, major rotamer C(CH_3)$_3$), 1.40 (9H, s, major rotamer C(CH_3)$_3$); δ_C (100 MHz, $CDCl_3$) 176.3, 175.1 (major and minor rotamer *C*O_2H),

152.7, 151.3 (major amd minor rotamer $CO_2{}^tBu$), 95.2 (major and minor rotamer $C(CH_3)_2$), 82.9, 81.6 (major and minor rotamer $C(CH_3)_3$), 66.2, 65.8 (major and minor rotamer $CHCH_2$), 59.1 (major and minor rotamer $CHCH_2$), 28.3 (major and minor rotamer $C(CH_3)_3$), 24.9, 24.3 (major and minor rotamer $C(CH_3)_2$); *m/z* (ESI+) 268.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{11}H_{19}NO_5Na$: 268.1155, found 268.1147 $[M+Na]^+$. Spectroscopic data are consistent with that previously reported.[24]

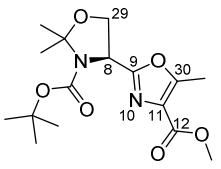
Dipeptide 4.27



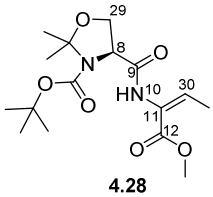
A solution of the acid **4.26** (15.5 g, 62.9 mmol) and HOBt (88%, 1.45 g, 9.44 mmol) in EtOH (300 mL) was stirred at room temperature for 15 minutes. To this was added a solution of L-threonine methyl ester hydrochloride (11.7 g, 69.2 mmol) in EtOH (100 mL) and the mixture was cooled to 0-5 °C. *N*-Methylmorpholine (22.0 mL, 202.3 mmol) was added over 5 minutes, and the reaction was stirred at 0-5 °C for 15 minutes. EDCI (14.4 g, 75.5 mmol) was added in one portion, and the reaction mixture was allowed to reach room temperature overnight. The reaction was acidified with pH 2 buffer and was reduced *in vacuo*. EtOAc (250 mL) was added, the mixture was vigorously agitated and the phases were separated. The organic phase was washed with pH 2 buffer (100 mL), water (100 mL), saturated aqueous $NaHCO_3$ solution, and saturated aqueous NaCl solution (100 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the dipeptide **4.27** as an orange crystalline solid (19.1 g, 53 mmol, 84 %); m.p. 77 - 80 °C; $[\alpha]_D^{25}$ -61.0 (c = 1.1, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3437 (O-H), 1735 (ester C=O), 1655 (amide C=O), 1524 (N-H); δ_H (500 MHz, $CDCl_3$) 7.27 (1H, s, major and minor rotamer N10-*H*), 4.55 (1H, dd, *J* 9.0, 2.0 Hz, major and minor rotamer C8-*H*), 4.49 - 4.00 (4H, m, major and minor rotamer C11-*H*, C29-H_2, C30-*H*), 3.73 (3H, s, OCH_3), 2.74 (1H, br. s., C30-O*H*), 1.85 – 1.36 (17H, m, major and minor rotamer $C(CH_3)_3$, $C(CH_3)_2$), 1.17 (3H, d, *J* 6.5

Hz, major and minor rotamer C30-C*H*$_3$); δ_C (125 MHz, $CDCl_3$) 173.4, 171.0 (major and minor rotamer *C9*, *C12*), 153.1, 151.8 (major and minor rotamer *C*O_2^{t}Bu), 95.12, 94.6 (major NS minor rotamer *C*$(CH_3)_2$), 81.4 (major and minor rotamer *C*$(CH_3)_3$), 68.2, 67.7 (major and minor rotamer *C*8-H), 67.1, 65.5 (major and minor rotamer *C29*-H_2), 60.8, 60.2 (major and minor rotamer *C30*-H), 57.4 (major and minor rotamer *C11*-H), 52.5 (major and minor rotamer O*C*H_3), 28.2, 26.6 (major and minor rotamer C(*C*H_3)$_3$, C(*C*H_3)$_2$), 19.9 (major and minor rotamer C30-*C*H_3); *m/z* (ESI+) 383.1 ([M+Na] 100%), 361.1 ([M+H] 60%); HR-ESIMS: calculated for $C_{16}H_{28}N_2O_7Na$: 383.1789, found 383.1785 $[M+Na]^+$.

Oxazole Methyl Ester 4.10 and Alkene 4.28



4.10



4.28

Method modified from literature procedure of Doi *et al.*[10] A solution of the dipeptide **4.27** (15.9 g, 44.2 mmol) in dry CH_2Cl_2 (220 mL) was cooled to 0 – 5 °C under a nitrogen atmosphere. Diisopropylethylamine (30.0 mL, 176.4 mmol) and dry DMSO (16.0 mL, 225.3 mmol) were added sequentially, followed by the addition of SO_3.pyr over 5 minutes. The reaction mixture was stirred at 0 – 5 °C for 3 hours, then poured into ice cold pH 2 buffer (200 ml) and stirred for 5 minutes. The phases were separated and the aqueous phase was further extracted with dichloromethane (2 x 10 mL). The combined organic extracts were dried over Na_2SO_4 and the ketone obtained by silica chromatography (50% EtOAc: pet ether) as a yellow oil (10.7 g, 29.8 mmol, 68 %). This was used immediately.

Oxazole **4.10** prepared according to the method of Bagley *et al.*[25] Iodine (14.9 g, 58.6 mmol) and triethylamine (16.8 mL, 120.2 mmol) were added sequentially to an ice cold solution of triphenylphosphine (15.4 g, 58.6 mmol) in CH_2Cl_2 (150 mL) under a nitrogen atmosphere. A solution of the ketone (10.5 g, 29.3 mmol) in

CH_2Cl_2 was added dropwise and the reaction mixture was allowed to reach room temperature overnight. The mixture was concentrated *in vacuo* and the residue was partitioned between EtOAc (150 mL) and pH 2 buffer (100 mL). The phases were separated and the organic phase was washed with pH 2 buffer (100 mL), water (100 mL) and saturated aqueous NaCl solution (100 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The oxazole was obtained by silica chromatography (25% to 50% EtOAc: pet. ether) as a pale yellow solid (4.15 g, 12.2 mmol, 28% over 2 steps); m.p. 60 - 63 °C; $[\alpha]_D^{25}$ -87.1 (c = 1.1, $CHCl_3$); υ_{max}/cm^{-1} (neat) 1698 (C=O), 1609 (C=N); δ_H (400 MHz, $CDCl_3$) 5.15 - 5.07 (1H, m, minor rotamer C8-*H*), 4.99 (1H, dd, *J* 6.0 Hz, 3.0 Hz, major rotamer C8-*H*), 4.25 - 4.04 (2H, m, major and minor rotamer C29-H_2), 3.88 (3H, s, major rotamer OCH_3), 3.85 (3H, br. s., minor rotamer OCH_3), 2.60 (3H, s, major rotamer C30-CH_3), 2.57 (3H, br. s., minor rotamer C30-CH_3), 1.71 (3H, s, major rotamer C(CH_3)$_2$), 1.67 (3H, br. s., minor rotamer C(CH_3)$_2$), 1.58 - 1.52 (3H, m, minor rotamer C(CH_3)$_2$), 1.46 (9H, br. s., minor rotamer C(CH_3)$_3$), 1.28 (9H, s, major rotamer C(CH_3)$_3$); δ_C (100 MHz, $CDCl_3$) 162.6 (major and minor rotamer *C10*), 161.1 (major and minor rotamer *C9*), 156.2 (major and minor rotamer *C30*), 151.2 (major and minor rotamer *C*$O_2{}^t$Bu), 127.5 (major and minor rotamer *C11*), 65.1 (major and minor rotamer *C*$(CH_3)_2$), 81.1, 80.5 (major and minor rotamer *C*$(CH_3)_3$), 67.5, 67.4 (major and minor rotamer *C29*-H_2), 55.1 (major and minor rotamer *C8*-H), 52.3, 51.9 (major and minor rotamer O*C*H_3), 28.3 (major and minor rotamer C(*C*H_3)$_3$), 24.9, 24.1 (major and minor rotamer C(*C*H_3)$_2$), 11.9 (major and minor rotamer C30-*C*H_3); *m/z* (ESI+) 363.1 ([M+Na] 100%), 341.1 ([M+H] 60%); HR-ESIMS: calculated for $C_{16}H_{24}N_2O_6Na$: 363.1527, found 363.1526 $[M+Na]^+$. This compound has not been previously reported. In some cases this method led to the formation of a significant amount of the elimination product **4.28** (~20 % of isolated product, inseperable from the desired oxazole **4.10**), identifiable by ^1H spectroscopy; δ_H (400 MHz, $CDCl_3$) 6.83 (1H, q, *J* 7.0 Hz, C30-*H*), 1.79 (3H, d, *J* 7.0 Hz, C30-CH_3).

The oxazole **4.28** was also prepared using (diethylamino)sulfur trifluoride. (Diethylamino)sulfur trifluoride (3.6 mL, 27.2 mmol) was added dropwise to a solution of the alcohol in dry CH_2Cl_2 (190 mL) at -78 °C under a nitrogen atmosphere. The recation mixture was stirred at -78 °C for 1.5 hours, and at room temperature for a further 30 minutes. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (200 mL) and was stirred for 15 minutes. The phases were separated and the aqueous phase was further extracted with CH_2Cl_2 (2 x 25 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated *in vacuo* to give a mix of the oxazoline and the undesired alkene **4.28** (~3: 1 oxazoline: alkene). Purification by silica chromatography (50% EtOAc: pet. ether) gave the oxazoline (1.0 g, 2.9 mmol, 15 %), which was used immediately.

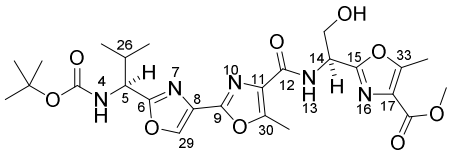
1, 8-Diazabicyclo[5.4.0]undec-7-ene (0.52 mL, 3.48 mmol) was added dropwise to a solution of the oxazoline (1.0 g, 2.9 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C under a nitrogen atmosphere. After 10 minutes, $BrCCl_3$ (0.40 mL, 3.8 mmol) was added dropwise, and the reaction was allowed to reach room temperature overnight. The reaction mixture was acidified with pH 2 buffer, and the phases were separated. The aqueous phase was further extracted with CH_2Cl_2 (2 x 25 mL) and the combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. The oxazole was obtained by silica chromatography (50% EtOAc: pet. ether) as a clear oil that crystallised on standing. All characterisation data was identical to the previously prepared sample.

Asymmetric Dihydroxylation of Alkene 4.28

In cases where the oxazoline and undesired alkene were not separated by silica chromatography, an inseparable mixture of the oxazole **4.10** and alkene **4.28** was obtained. The alkene was removed using a method modified from literature procedure of a Sharpless *et al.*[26] $K_3[Fe(CN)_6]$ (2.01 g, 6.13 mmol), K_2CO_3 (0.85 g, 6.13 mmol), $(DHQD)_2PHAL$ (0.02 g, 0.02 mmol) and methane sulphonamide (0.19

g, 2.04 mmol) were added to a solution of the mixture of oxazole **4.10** and alkene **4.28** (2.10 g; approximately 0.7 g, 2.04 mmol alkene **4.28**) in ^{t}BuOH:H_2O (1: 1, 50 mL) at room temperature. After 5 minutes, $K_2OsO_4 \cdot 2H_2O$ (0.008 g, 0.02 mmol) was added and the reaction mixture was stirred at room temperature for 24 hours. Sodium sulfite (~0.1 g) was added and the mixture was stirred for a further 30 minutes. The phases were separated and the aqueous phase was further extracted with EtOAc (3 x 10 mL). The combined organic phases were washed with pH 2 buffer (10 mL), water (10 mL) and saturated aqueous $NaHCO_3$ solution (10 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Silica chromatography (50 % EtOAc: pet. ether) gave only the desired oxazole **4.10** (0.67 g, 1.98 mmol)**.** All characterisation data was consistent to that previously obtained.

Pentapeptide 4.30

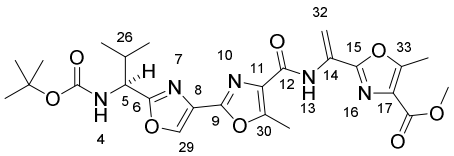


Oxazole methyl ester **4.10** (0.67 g, 2.0 mmol) was added to methanolic hydrochloric acid solution (4 M, 3 mL) and the reaction mixture was stirred at room temperature for 18 hours. The solvent was removed *in vacuo* to give the crude deprotected amine as the hydrochloride salt, **4.29**, which was used without further purification.

HOBt (88 %, 0.03 g, 0.19 mmol) was added to a solution of the bisoxazole (0.46 g, 1.3 mmol) in EtOH (4 mL) and the mixture stirred at room temperature for 15 minutes. A solution of the crude amine (2.0 mmol) in EtOH (3 mL) was added and the mixture cooled to 0 °C. *N*-Methylmorpholine (0.58 mL, 5.30 mmol) was added and the solution was stirred at 0-5 °C for 15 minutes. EDCI (0.29 g, 1.5 mmol) was added and the reaction was stirred to room temperature overnight. The reaction was acidified with pH 2 buffer and concentrated *in vacuo*. The residue was partitioned between EtOAc (10 mL) and pH 2 buffer (10 mL) and the phases were separated. The aqueous phase was further extracted with EtOAc (3 x 2 mL) and the combined

organic phases were washed with saturated aqueous NaCl solution (10 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The pentapeptide **4.30** was obtained by silica chromatography (EtOAc) as yellow solid (0.50 g, 0.77 mmol, 64 %); m.p. 104 - 105 °C; $[\alpha]_D^{25}$ -28.6 (c = 0.54, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3320 (NH), 2972 (OH), 1726 (acid C=O), 1683 (amide C=O), 1531 (NH); δ_H (400 MHz, $CDCl_3$) 8.15 (1H, s, C29-*H*), 7.82 (1H, d, *J* 8.5 Hz, N13-*H*), 5.46 (1H, dt, *J* 9.0, 4.5 Hz, C14-*H*), 5.41 (1H, d, *J* 9.0 Hz, N4-*H*), 4.86 – 4.79 (1H, m, C5-*H*), 4.23 (1H, dd, *J* 11.5, 4.5 Hz, CH_2OH), 4.05 (1H, dd, *J* 11.5, 4.5 Hz, CH_2OH), 3.90 (3H, s, OCH_3), 3.32 - 3.36 (1H, m, CH_2OH), 2.71 (3H, s, C30-CH_3), 2.61 (3H, s, C33-CH_3), 2.26 - 2.17 (1H, m, C26-*H*), 1.43 (9H, s, C(CH_3)$_3$), 0.97 (2H, d, *J* 7.0 Hz, C26(CH_3)$_2$), 0.94 (3H, d, *J* 6.5 Hz, C26(CH_3)$_2$); δ_C (150 MHz, $CDCl_3$) 165.8 (*C6*), 162.4 (*C*O_2Me), 161.5 (*C12*), 159.8 (*C15*), 157.0 (*C33*), 155.4 (*C*O_2^{t}Bu), 154.0 (*C30*), 152.2 (*C9*), 138.6 (*C29*-H), 129.8 (*C8*), 159.6 (*C11*), 127.4 (*C17*), 80.0 (*C*$(CH_3)_3$), 63.3 (*C*H_2OH), 54.4 (*C5*), 52.0 (O*C*H_3), 48.5 (*C14*), 33.0 (*C26*), 28.3 (C(*C*H_3)$_3$), 18.8, 18.1 (C26(*C*H_3)$_2$), 12.1 (C33-*C*H_3), 11.8 (C30-*C*H_3); *m/z* (ESI+) 570.2 ([M+Na] 100%); HR-ESIMS: calculated for $C_{25}H_{33}N_5O_9Na$: 570.1270, found 570.2172 $[M+Na]^+$.

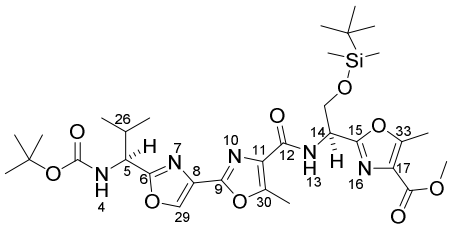
Alkene 4.31



Method modified from literature procedure of Zarantonello *et al.*[27] Lawesson's reagent (0.07 g, 0.18 mmol) was added to a solution of the alcohol **4.30** (0.10 g, 0.18 mmol) in dry toluene (3 mL) under a nitrogen atmosphere. The reaction was heated to reflux for 1 hour. After cooling to room temperature, the reaction mixture was concentrated *in vacuo* and purified by silica chromatography to give the alkene product **4.31** as an yellow oil (0.03 g, 0.057 mmol, 31 %); $[\alpha]_D^{25}$ -12.2 (c = 0.35, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 9.26 (1H, s, N13-*H*), 8.28 (1H, s, C29-*H*), 6.56 (1H, s, C32-H_2), 5.79 (3H, s, C32-H_2), 5.35 (1H, d, *J* 9.5 Hz, N4-*H*), 4.85 (1H, dd, *J* 9.0,

7.0 Hz, C5-*H*), 3.94 (3H, s, OCH_3), 2.76 (3H, s, C30-H_3), 2.69 (3H, s, C33-H_3), 2.31 – 2.19 (1H, m, C26-*H*), 1.45 (9H, s, C(CH_3)$_3$), 0.98 (3H, d, *J* 6.5 Hz, C26-(CH_3)$_2$), 0.95 (3H, d, *J* 6.5 Hz, C26-(CH_3)$_2$).

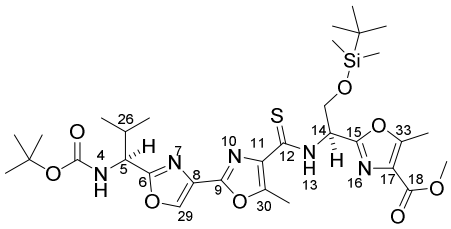
Pentapeptide Silyl Ether 4.32



Method modified from literature procedure of McKeever *et al*.[28] Imidazole (0.19 g, 2.80 mmol) was added in one portion to a solution of the alcohol **4.30** (0.75 g, 1.41 mmol) in dry DMF (6.5 mL) under an atmosphere of nitrogen at 0 °C. The reaction mixture was stirred at 0 °C for 5 minutes and ^{t}BuMe$_2$SiCl (0.32 g, 2.10 mmol) was added in one portion. The ice bath was removed and the reaction stirred at room temperature for 3 hours. The reaction mixture was diluted with EtOAc (10 mL) and H_2O (40 mL), and the phases were separated. The aqueous phase was further extracted with EtOAc (5 x 10mL). The combined organic extracts were washed with water (5 x 10 mL) and saturated aqueous NaCl solution (10 mL), dried over $NaSO_4$ and concentrated *in vacuo*. The protected product **4.31** was obtained by silica chromatography (1:1 EtOAc: pet. ether) as a bright white crystalline solid (0.57 g, 0.86 mmol, 62 %); m.p. 57 - 58 °C; $[\alpha]_D^{25}$ -1.05 (c = 0.37, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3319 (NH), 1716 (ester C=O), 1672 (amide C=O), 1506 (NH), 836 (Si-C); δ_H (400 MHz, $CDCl_3$) 8.16 (1H, s, *C29*-H), 7.74 (1H, d, *J* 8.5 Hz, N13-*H*), 5.42 (1H, dt, *J* 8.5, 5.0 Hz, C14-*H*), 5.29 (1H, d, *J* 9.0 Hz, N4-*H*), 4.85 (1H, dd, *J* 8.5, 6.0 Hz, C5-*H*), 4.12 (1H, dd, *J* 10.0, 4.5 Hz, CH_2OH), 4.00 (1H, dd, *J* 10.0, 5.5 Hz, CH_2OH), 3.90 (3H, s, OCH_3), 2.71 (3H, s, C30-CH_3), 2.61 (3H, s, C33-CH_3), 2.28 - 2.20 (1H, m, C26-*H*), 1.45 (9H, s, OC(CH_3)$_3$), 0.98 - 0.94 (6H, m, C26(CH_3)$_2$), 0.85 (9H, s, SiC(CH_3)$_3$), 0.02 (3H, s, SiCH_3), 0.00 (3H, s, SiCH_3); δ_C (100 MHz, $CDCl_3$) 165.6 (*C6*), 162.7 (*C*O_2Me), 161.3 (*C12*), 160.2 (*C15*), 156.6 (*C33*), 155.3 (*C*O^{t}Bu), 153.8

(*C30*), 152.0 (*C9*), 138.5 (*C29*-H), 130.1 (*C8*), 129.7 (*C11*), 127.6 (*C17*), 80.1 ($C(CH_3)_3$), 64.3 (C14-CH_2), 54.3 (C*5*), 51.9 (CO_2CH_3), 48.9 (C*13*), 33.0 (*C26*), 28.3 ($C(CH_3)_3$), 25.7 ($SiC(CH_3)_3$), 18.7, 18.0 ($C26(CH_3)_2$), 17.9 ($SiC(CH_3)_3$), 12.0, 11.8 (C30-CH_3, C33-CH_3), -5.4 ($Si(CH_3)_2$); *m/z* (ESI+) 684.3 ([M+Na] 100%); HR-ESIMS: calculated for $C_{31}H_{47}N_5O_9SiNa$: 684.3035, found 684.3023 $[M+Na]^+$.

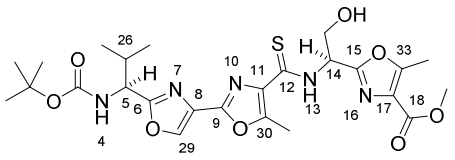
Thioamide Silyl Ether 4.33



Method modified from literature procedure of McKeever *et al*.[28] The silyl ether **4.32** (0.50 g, 0.76 mmol) was dissolved in dry THF (10 mL) and dry toluene (6.5 mL) under nitrogen and Lawesson's reagent (0.34 g, 0.83 mmol) was added. The reaction was heated to reflux under a nitrogen atmosphere for 44 hours, during which Lawesson's reagent (1.08 g, 2.67 mmol) and dry THF (15 mL) were added in 3 equal portions after 18, 24 and 40 hours. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The residue was partitioned between EtOAc (20 mL) and aqueous $NaHCO_3$ solution (1: 1 saturated $NaHCO_3$: water, 20 mL). The separated aqueous phase was further extracted with EtOAc (3 x 5 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. The protected thioamide **4.33** was obtained by silica chromatography (5 % acetone: toluene) as a bright yellow oil (0.39 g, 0.58 mmol, 76 %); $[\alpha]_D^{22}$ +13.3 (c = 1.2, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3320 (N-H), 1685 (ester C=O), 1504 (N-H), 1252 (C=S), 833 (Si-C); δ_H (400 MHz, $CDCl_3$) 9.52 (1H, d, *J* 8.5 Hz, N13-*H*), 8.15 (1H, s, C29-*H*), 6.01 (1H, dt, *J* 8.5, 4.5 Hz, C14-*H*), 5.30 (1H, d, *J* 9.0 Hz, N4-*H*), 4.83 (1H, dd, *J* 9.0, 6.5 Hz, C5-*H*), 4.22 (1H, dd, *J* 10.5, 4.0 Hz, C14-CH_2), 4.07 (1H, dd, *J* 10.5, 5.5 Hz, C14-CH_2), 3.88 (3H, s, OCH_3), 2.88 (3H, s, C30-CH_3), 2.59 (3H, s, C33-CH_3), 2.28 - 21 (1H, m, C26-*H*), 1.43 (9H, s, $C(CH_3)_3$), 0.96 (2H, d, *J* 7.0 Hz, $C26(CH_3)_2$),

0.94 (3H, d, *J* 6.5 Hz, C26(C*H*$_3$)$_2$), 0.84 - 0.87 (9H, m, SiC(C*H*$_3$)$_3$), 0.04 (3H, s, SiC*H*$_3$), -0.01 (3H, s, SiC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 186.4 (*C12*), 165.6 (*C6*), 162.6 (*C*O_2Me), 159.5 (*C15*), 156.7 (*C33*), 155.4 (*C*O^{t}Bu, *C30*), 150.6 (*C9*), 138.7 (*C29*-H), 134.2 (*C11*), 129.9 (*C8*), 127.6 (*C17*), 80.1 (*C*$(CH_3)_3$), 63.6 (C14-*C*H_2), 54.3 (*C5*), 53.4 (*C14*), 51.9 (CO_2CH$_3$), 32.9 (*C26*), 28.3 (OC(*C*H$_3$)$_3$), 25.7 (SiC(*C*H$_3$)$_3$), 18.7, 18.1 (C26(*C*H$_3$)$_2$), 18.0 (Si*C*$(CH_3)_3$), 13.8 (C30-*C*H$_3$), 12.0 (C33-*C*H$_3$), -5.6 (Si(*C*H$_3$)$_2$); *m/z* (ESI+) 700.3 ([M+Na] 100%); HR-ESIMS: calculated for $C_{31}H_{47}N_5O_8SSiNa$: 700.2807, found 700.2790 $[M+Na]^+$.

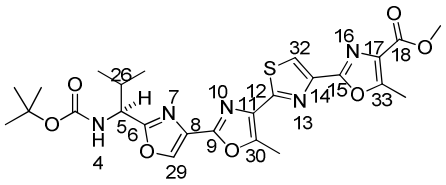
Thioamide 4.34



Bu_4NF (1M solution in THF, 1.0 mL, 1.0 mmol) was added to the thioamide silyl ether **4.33** (0.34 g, 0.50 mmol) under a nitrogen atmosphere at 0 °C. The mixture was stirred at 0 °C for 1 hour to give a brown solution. The reaction mixture was concentrated *in vacuo* and the residue was partitioned between EtOAc (5 mL) and water (2 mL). The phases were separated, and the organic phase was washed with water (2 x 2 mL), saturated aqueous NaCl solution (2 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give a yellow oil. The alcohol **4.34** was obtained by silica chromatography (50% EtOAc: petrol) as a yellow glassy solid (0.24 g, 0.45 mmol, 90 %); m.p. 127 - 129 °C; $[\alpha]_D^{27}$ -18.3 (c = 0.36, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3340 (NH), 2972 (OH), 1708 (ester C=O), 1519 (NH), 1249 (C=S); δ_H (400 MHz, $CDCl_3$) 9.56 (1H, d, *J* 8.0 Hz, N13-*H*), 8.17 (1H, s, C29-*H*), 6.15 (1H, dt, *J* 8.5, 4.5 Hz, C14-*H*), 5.53 (1H, d, *J* 9.0 Hz, N4-*H*), 4.81 (1H, dd, *J* 8.5, 6.5 Hz, C5-*H*), 4.31 (1H, dd, *J* 11.5, 4.5 Hz, C*H*$_2$OH), 4.16 (1H, dd, *J* 11.5, 4.0 Hz, C*H*$_2$OH), 3.86 (3H, s, OC*H*$_3$), 2.85 (3H, s, C30-C*H*$_3$), 2.57 (3H, s, C33-C*H*$_3$), 2.27 – 2.14 (1H, m, C26-*H*), 1.40 (9H, s, C(C*H*$_3$)$_3$), 0.96 (3H, d, *J* 7.0 Hz, C26(C*H*$_3$)$_2$), 0.92 (3H, d, *J* 6.5 Hz,

C26(CH_3)$_2$); δ_C (100 MHz, $CDCl_3$) 186.5 (*C12*), 165.6 (*C6*), 162.3 (*C18*), 159.2 (*C15*), 157.0 (*C33*), 155.6, 155.5 (*C30*, $CO_2{}^tBu$), 150.8 (*C9*), 138.9 (*C29*-H), 134.1 (*C11*), 129.6 (*C8*), 127.4 (*C17*), 80.0 ($C(CH_3)_3$), 62.5 (CH_2OH), 54.5 (*C5*), 53.2 (*C14*), 51.9 (OCH_3), 32.9 (*C*26), 29.3 ($C(CH_3)_3$), 18.8, 18.1 (C26(CH_3)$_2$), 13.9 (C30-CH_3), 12.0 (C33-CH_3); *m/z* (ESI+) 586.2 ([M+Na] 100%); HR-ESIMS: calculated for $C_{25}H_{33}N_5O_8SNa$: 586.1942, found 586.1950 $[M+Na]^+$.

Pentapeptide 4.35

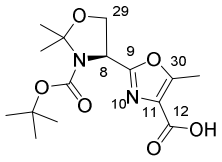


A solution of the thioamide **4.34** (0.24 g, 0.46 mmol) in dry CH_2Cl_2 (5 mL) was cooled to -78 °C under a nitrogen atmosphere. (Diethylamino)sulfur trifluoride (0.11 mL, 0.80 mmol) was added dropwise and the reaction mixture was stirred at -78 °C for 1 hour, then to room temperature over 30 minutes. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (5 mL) and stirred for 15 minutes. The biphasic mixture was separated and the aqueous phase was extracted with CH_2Cl_2 (2 x 5 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo* to give the crude thiazoline as a bright orange oil. This was used without further purification.

The thiazole was prepared using a method modified from the literature procedure of Videnov *et al*.[29] The solution of the crude thiazoline (0.46 mmol) in acetonitrile (1.5 mL), CCl_4 (1.1 mL), pyridine (1.5 mL) was cooled to 0 °C under a nitrogen atmosphere and 1, 8-Diazabicyclo[5.4.0]undec-7-ene (0.30 mL, 2.00 mmol) was added dropwise. The reaction was allowed to reach room temperature overnight. The resulting brown suspension was partitioned between pH 2 buffer (5 mL) and CH_2Cl_2 (5 mL) and the phases separated. The aqueous phase was further extracted with CH_2Cl_2 (3 x 5 mL), and the combined organic phases were dried over Na_2SO_4 and

concentrated *in vacuo* to give the crude thiazole. This was purified by silica chromatography (50 % EtOAc: pet. ether) to give the thiazole **4.35** as a bright white solid (0.11 g, 0.20 mmol, 45 %); m.p. 206 - 209 °C; $[\alpha]_D^{26}$ -3.57 (c = 0.49, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3349 (NH), 1708 (ester C=O), 1686 (amide C=O), 1523 (NH); δ_H (400 MHz, $CDCl_3$) 8.22 (1H, s, C29-*H*), 8.09 (1H, s, C32-*H*), 5.32 (1H, d, *J* 9.0 Hz, N4-*H*), 4.85 (1H, dd, *J* 8.5, 6.5 Hz, C5-*H*), 3.97 - 3.92 (3H, m, OCH_3), 2.88 (3H, s, C30-CH_3), 2.74 (3H, s, C33-CH_3), 2.22 (1H, s, C26-H), 1.44 (9H, s, C(CH_3)$_3$), 0.96 (3H, d, *J* 7.0 Hz, C26(CH_3)$_2$), 0.94 (8H, d, *J* 7.0 Hz, *M11*); δ_C (150 MHz, $CDCl_3$) 165.6 (*C6*), 162.7 (*C18*), 162.0 (*C12*), 156.5 (*C33*), 155.4, 155.3 (*C*O_2^{t}Bu, *C15*), 153.3 (*C9*), 148.2 (*C30*), 143.6 (*C14*), 138.6 (*C29*-H), 130.8 (*C11*), 130.0 (*C8*), 128.4 (*C17*), 120.5 (*C32*-H), 80.1 (*C*$(CH_3)_3$), 54.4 (*C5*-H), 52.1 (CO_2CH_3), 32.4 (*C26*-H), 28.3 (C(*C*H_3)$_3$), 18.8, 18.1 (C26(*C*H_3)$_2$), 12.2, 12.1 (C30-CH_3, C33-CH_3); *m/z* (ESI+) 566.2 ([M+Na] 100%); HR-ESIMS: calculated for $C_{25}H_{29}N_5O_7SNa$: 566.1680, found 566.1679 $[M+Na]^+$.

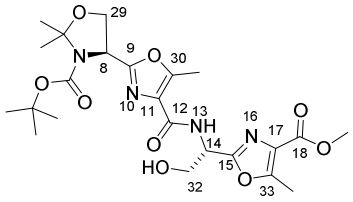
Oxazole Acid 4.36



Lithium hydroxide (0.26 g, 11.1 mmol) was added to a solution of methyl ester **4.10** (0.94 g, 2.76 mmol) in THF (6 mL) and water (6 mL), and the biphasic solution was stirred at room temperature for 4 hours. The reaction was acidified with pH 2 buffer and the THF removed *in vacuo*. The residue was extracted with EtOAc (4 x 10 mL). The combined organic extracts were washed with saturated aqueous NaCl solution (10 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the acid **4.36** as bright white solid (0.85 g, 2.6 mmol, 94 %); m.p. 116 - 118 °C; $[\alpha]_D^{22}$ = -85.2 (c = 0.2, $CHCl_3$); υ_{max}/cm^{-1} (neat) 2980 (O-H), 1738 (acid C=O), 1702 (carbonyl C=O); δ_H (500 MHz, $CDCl_3$) 5.19 - 5.05 (1H, m, major and minor rotamer C8-*H*), 4.31 - 4.10 (2H, m, major and minor rotamer C29-H_2), 2.66 (3H, s, major rotamer C30-CH_3),

2.63 (3H, br. s., minor rotamer C30-CH_3), 1.75 (3H, s, major rotamer C(CH_3)$_2$), 1.70 (3H, br. s., minor rotamer C(CH_3)$_2$), 1.60 (3H, s, major rotamer C(CH_3)$_2$), 1.57 (3H, br. s., minor rotamer C(CH_3)$_2$), 1.50 (9H, br. s., minor rotamer C(CH_3)$_3$), 1.32 (9H, s, major rotamer C(CH_3)$_3$); δ_C (125 MHz, $CDCl_3$) 165.0, 164.6 (major and minor rotamer *C12*), 161.5, 161.1 (major and minor rotamer *C9*), 157.21 (major and minor rotamer *C30*), 152.1, 151.2 (major and minor rotamer *C*O$_2$^{t}Bu), 127.1 (major and minor rotamer *C11*), 95.2, 94.7 (major and minor rotamer *C*(CH$_3$)$_2$), 81.4, 80.7 (major and minor rotamer *C*(CH$_3$)$_3$), 64.5, 67.3 (major and minor rotamer *C29*-H$_2$), 55.0 (*C8*), 28.3, 28.2 (major and minor rotamer C(*C*H$_3$)$_3$); 26.4, 25.3, 24.9, 24.1 (major and minor rotamer C(*C*H$_3$)$_2$), 12.1 (major and minor rotamer C30-*C*H$_3$); *m/z* (ESI-) 325.1 ([M-H] 100%); HR-ESIMS: calculated for $C_{15}H_{21}N_2O_6$: 325.1405, found 325.1401 [M-H]$^-$.

Tetrapeptide 4.37

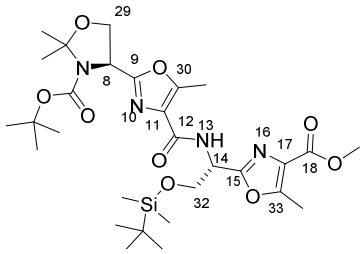


Oxazole methyl ester **4.10** (0.94 g, 2.76 mmol) was dissolved in a solution of methanolic hydrochloride (4 M, 7 mL) and stirred at room temperature for 18 hours. The reaction mixture was concentrated *in vacuo* to give the crude deprotected amine as the hydrochloride salt, which was used without further purification assuming quantitative yield.

A solution of the oxazole acid **4.36** (0.82 g, 2.5 mmol) and HOBt (88 %, 0.06 g, 0.52 mmol) in EtOH (5 mL) was stirred at room temperature for 15 minutes. To this was added a solution of the crude amine (2.76 mmol) in EtOH (10 mL) and the reaction mixture was cooled to 0-5 °C. *N*-Methylmorpholine (0.9 mL, 8.3 mmol) was added and the reaction was stirred at 0-5 °C for 15 minutes. EDCI (0.57 g, 2.98 mmol) was added, and the reaction mixture allowed to reach room temperature overnight. The

reaction mixture was acidified with pH 2 buffer, and concentrated *in vacuo*. The residue was partitioned between pH 2 buffer (5 mL) and EtOAc (10 mL) and the phases were separated. The organic phase was washed with pH 2 buffer (5 mL), water (5 mL), saturated aqueous $NaHCO_3$ solution (5 mL) and saturated aqueous NaCl solution (5 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The tetrapeptide **4.37** was obtained by silica chromatography (EtOAc) as a white crystalline solid (0.77 g, 1.51 mmol, 61 %); m.p. 88 - 91 °C; $[\alpha]_D^{22}$ +1.48 (c = 0.37, $CHCl_3$); υ_{max}/cm^{-1} (neat) 2982 (O-H), 1703 (ester C=O), 1622 (amide C=O); δ_H (500 MHz, $CDCl_3$) 7.69 (1H, d, *J* 7.5 Hz, major and minor rotamer N13-*H*), 5.41 (1H, br. s., major and minor rotamer C14-*H*), 5.10 - 4.90 (1H, m, major and minor rotamer C8-*H*), 4.26 - 4.00 (4H, m, major and minor rotamer C29-H_2, C31-H_2,), 3.91 (3H, s, major and minor rotamer OCH_3), 2.62 (6H, s, major and minor rotamer C30-CH_3, C33-CH_3), 1.73 (3H, br. s., major rotamer C(CH_3)$_2$), 1.70 (3H, br. s., minor rotamer C(CH_3)$_2$), 1.60 (3H, br. s., major rotamer C(CH_3)$_2$), 1.57 (3H, br. s., minor rotamer C(CH_3)$_2$), 1.49 (9H, br. s., minor rotamer C(CH_3)$_3$), 1.32 (10H, s, major rotamer C(CH_3)$_3$); δ_C (125 MHz, $CDCl_3$) 162.4, 161.7 (major and minor rotamer *C12, C18*), 160.4, 159.9, 157.2 (major and minor rotamer *C9*, *C15*, *C30*, *C33*), 153.7 (major and minor rotamer *C*$O_2{}^t$Bu), 128.7 (major and minor rotamer *C11*, *C17*), 95.1, 94.6 (major and rotamer *C*(CH_3)$_2$), 81.2, 80.6 (major and minor rotamer *C*(CH_3)$_3$), 67.3, 67.0 (major rotamer *C29*-H_2, minor rotamer *C29*-H_2), 63.5 (major and minor rotamer *C32*-H_2), 55.0 (*C8*-H), 52.3 (major and minor rotamer OCH_3), 48.3 (C14-H) 28.3, 28.2 (major and minor rotamer C(*C*H_3)$_3$), 26.4, 25.4, 25.0, 24.2 (major and minor rotamer C(*C*H_3)$_2$), 12.1, 11.7 (C30-*C*H_3, C33-*C*H_3); *m/z* (ESI+) 531.2 ([M+Na] 100%); HR-ESIMS: calculated for $C_{23}H_{32}N_4O_9Na$: 531.2061, found 531.2057 $[M+Na]^+$.

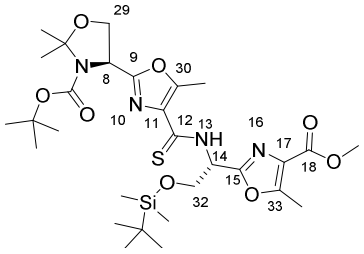
Tetrapeptide Silyl Ether 4.38



Imidazole (0.21 g, 3.0 mmol was added in one portion to a solution of alcohol **4.37** (0.77 g, 1.51 mmol) in dry DMF (7.0 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 5 minutes and $^{t}BuMe_2SiCl$ (0.34 g, 2.27 mmol) was added in one portion and the ice bath removed. The reaction mixture was stirred at room temperature overnight, then diluted with EtOAc (20 mL) and water (80 mL). The phases were separated and the aqueous phase was further extracted with EtOAc (5 x 10 mL). The combined organic extracts were washed with water (5 x 20 mL) and saturated aqueous NaCl solution (20 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The protected alcohol **4.38** was obtained by silica chromatography (50 % EtOAc: pet. ether) as a clear colourless oil (0.57 g, 0.92 mmol, 61 %); $[\alpha]_D^{22}$ -50.4 (c = 2.0, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3414 (N-H), 1704 (ester C=O), 1632 (amide C=O), 1508 (N-H), 835 (Si-C); δ_H (400 MHz, $CDCl_3$) 7.81 - 7.52 (1H, m, major and minor rotamer N13-*H*), 5.36 (1H, dt, *J* 8.5 Hz, 4.0 Hz, major and minor rotamer C14-*H*), 5.10 - 4.89 (1H, m, major and minor rotamer C8-*H*), 4.10 - 4.26 (1H, m, major and minor rotamer C29-H_2), 4.26 - 4.10 (1H, m, major and minor rotamer C29-H_2), 4.09 (1H, dd, *J* 8.5, 3.0 Hz, major and minor rotamer C32-H_2), 3.96 (1H, dd, *J* 10.0, 4.5 Hz, major and minor rotamer C32-H_2), 3.90 - 3.85 (3H, m, major and minor rotamer OCH_3), 2.63 - 2.52 (6H, m, major and minor rotamer C30-CH_3, C33-CH_3), 1.73 (3H, br. s., major rotamer C(CH_3)$_2$), 1.70 (3H, br. s., minor rotamer C(CH_3)$_2$), 1.59 (3H, br. s., major rotamer C(CH_3)$_2$), 1.56 (3H, br. s., minor rotamer C(CH_3)$_2$), 1.49 (9H, br. s., minor rotamer OC(CH_3)$_3$), 1.29 (9H, br. s., minor rotamer OC(CH_3)$_3$), 0.83 -

0.79 (9H, m, Si(C(C*H*$_3$)$_3$), -0.02 (3H, s, Si(C*H*$_3$)$_2$), -0.05 (3H, s, Si(C*H*$_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 162.7 (*C18*), 161.5 (*C12*), 160.2, 159.9 (*C9*, *C15*), 156.5 (*C33*), 153.5 (*C30*), 151.2 (*C*O$_2$^{t}Bu), 128.9, 127.6 (*C11*, *C17*), 95.1 (*C*(CH$_3$)$_2$), 80.4 (*C*(CH$_3$)$_3$), 67.2 (*C29*-H$_2$), 64.4 (*C32*-H$_2$), 54.9 (*C8*-H), 51.9 (O*C*H$_3$), 49.0 (*C14*), 28.3, 28.2 (OC(*C*H$_3$)$_3$), 25.6 (SiC(*C*H$_3$)$_3$), 25.3, 24.3 (C(*C*H$_3$)$_2$), 17.7 (Si*C*(CH$_3$)$_3$), 11.9, 11.7 (C30-*C*H$_3$, C33-*C*H$_3$), -5.64 (Si(*C*H$_3$)$_2$); *m/z* (ESI+) 645.2 ([M+Na] 100%); HR-ESIMS: calculated for $C_{29}H_{46}N_4O_9SiNa$: 645.2926, found 645.2928 $[M+Na]^+$.

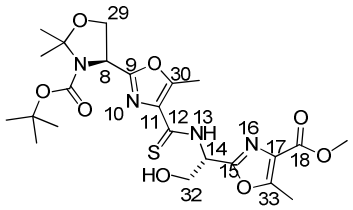
Tetrapeptide Thioamide Silyl Ether 4.39



The silyl ether **4.38** (1.15 g, 1.85 mmol) was dissolved in dry THF (7.5 mL) and dry toluene (15 mL) under nitrogen and Lawesson's reagent (0.60 g, 1.48 mmol) was added. The reaction was heated to reflux under a nitrogen atmosphere for 24 hours, with Lawesson's reagent (0.38 g, 0.94 mmol) added in one portion after 12 hours. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The residue was partitioned between EtOAc (20 mL) and aqueous $NaHCO_3$ solution (1:1 saturated $NaHCO_3$ solution: water, 20 mL). The separated aqueous phase was further extracted with EtOAc (3 x 5 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. The thioamide **4.39** was obtained by silica chromatography (5 % acetone: toluene) as a bright yellow oil (0.98 g, 1.53 mmol, 83 %); $[\alpha]_D^{22}$ -83.0 (c = 0.11, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3349 (NH), 1704 (ester C=O), 1505 (NH), 1253 (C=S), 836 (Si-C); δ_H (600 MHz, $CDCl_3$) 9.69 - 9.27 (1H, m, major and minor rotamer N13-*H*), 6.02 - 5.89 (1H, m, major and minor rotamer C14-*H*), 5.11 - 4.89 (1H, m, major and minor rotamer C8-*H*), 4.29 - 4.23 (1H, m, major and minor rotamer C32-*H*$_2$), 4.21 (1H, dd, *J* 10.5, 4.0 Hz, 1H, m, major and minor

rotamer C29-H_2), 4.12 (1H, dd, *J* 9.5, 2.5 Hz, 1H, m, major and minor rotamer C29-H_2), 4.09 - 4.04 (1H, m, 1H, m, major and minor rotamer C32-H_2), 3.90 (3H, s, 1H, m, major and minor rotamer OCH_3), 2.81 (3H, s, major rotamer C30-CH_3), 2.79 (3H, br. s., minor rotamer C30-CH_3), 2.60 (3H, s, major and minor rotamer C33-CH_3), 1.75 (3H, s, major rotamer C(CH_3)$_2$), 1.72 (3H, br. s., minor rotamer C(CH_3)$_2$), 1.61 (3H, s, major rotamer C(CH_3)$_2$), 1.58 (3H, br. s., minor rotamer OC(CH_3)$_2$), 1.52 - 1.48 (9H, m, minor rotamer OC(CH_3)$_3$), 1.31 (9H, s, major rotamer C(CH_3)$_3$), 0.83 (9H, s, SiC(CH_3)$_3$), 0.01 (3H, s, Si(CH_3)$_2$), -0.03 (3H, s, Si(CH_3)$_2$); δ_C (150 MHz, $CDCl_3$) 186.8 (*C12*), 162.6 (*C18*), 159.4, 158.9 (*C9*, *C15*), 156.6 (*C33*), 155.1 (*C30*), 151.1 (*C*O$_2$^{t}Bu), 133.5 (*C11*), 127.6 (*C17*), 95.1 (*C*(CH$_3$)$_2$), 81.2 (O*C*(CH$_3$)$_3$), 67.4 (*C29*-H$_2$), 63.8 (*C32*-H$_2$), 54.9 (*C8*-H), 53.6 (*C14*-H), 51.9 (O*C*H$_3$), 28.3 (OC(*C*H$_3$)$_3$), 25.6 (SiC(*C*H$_3$)$_3$), 25.4, 24.2 (C(*C*H$_3$)$_2$), 18.1 (Si*C*(CH$_3$)$_3$), 13.8 (C30-*C*H$_3$), 12.0 (C33-*C*H$_3$), -5.4 (Si(*C*H$_3$)$_2$); *m/z* (ESI+) 661.3 ([M+Na] 100%); HR-ESIMS: calculated for $C_{29}H_{46}N_4O_8SSiNa$: 661.2698, found 661.2689 $[M+Na]^+$.

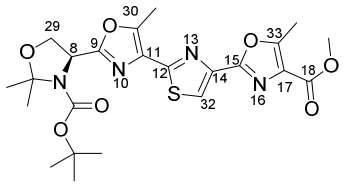
Thioamide Alcohol 4.40



Bu_4NF (1 M solution in THF, 2.5 mL, 2.5 mmol) was added to silyl ether **4.39** (0.80 g, 1.25 mmol) under a nitrogen atmosphere at 0 °C. The mixture was stirred at 0 °C for 1 hour to give a brown solution. The reaction mixture was concentrated *in vacuo* and the residue was partitioned between EtOAc (5 mL) and water (2 mL). The phases were separated, and the organic phase was washed with water (2 x 2 mL), saturated aqueous NaCl solution (2 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give a yellow oil. The alcohol **4.40** was obtained by silica chromatography (EtOAc) as a bright yellow oil (0.52 g, 0.99 mmol, 79 %); $[\alpha]_D^{27}$ -81.1 (c = 0.67, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3340 (NH), 1699 (ester C=O), 1615 (amide C=O), 1505

(NH), 1365 (C=S); δ_H (600 MHz, $CDCl_3$) 9.45 (1H, d, *J* 7.5 Hz, major and minor rotamer N13-*H*), 6.19 - 6.09 (1H, m, major and minor rotamer C14-*H*), 5.11 - 4.92 (1H, m, major and minor rotamer C8-*H*), 4.34 - 4.26 (1H, m, major and minor rotamer C32-H_2), 4.25 - 4.20 (1H, m, major and minor rotamer C29-H_2), 4.16 - 4.05 (2H, m, major and minor rotamer C29-H_2, C32-H_2), 3.91 (3H, s, major and minor rotamer OCH_3), 2.83 (3H, s, major rotamer C30-CH_3), 2.80 (3H, s, minor rotamer C30-CH_3), 2.62 (3H, s, major and minor rotamer C33-CH_3), 1.73 (3H, s, major rotamer C(CH_3)$_2$), 1.70 (3H, br. s., minor rotamer C(CH_3)$_2$), 1.60 (3H, s, major rotamer C(CH_3)$_2$), 1.57 (3H, br. s., minor rotamer C(CH_3)$_2$), 1.48 (9H, s, minor rotamer C(CH_3)$_3$), 1.32 (9H, s, major rotamer C(CH_3)$_3$); δ_C (125 MHz, $CDCl_3$) 186.9, 186.7 (major and minor rotamer *C12*), 162.4 (major and minor rotamer *C18*), 159.2, 158.7 (major and minor rotamer *C9*, *C15*), 157.2 (major and minor rotamer *C33*), 155.7, 155.4 (major and minor rotamer *C30*), 151.2 (major and minor rotamer *C*O$_2$^{t}Bu), 133.5, 133.4 (major and minor rotamer *C11*), 127.5 (major and minor rotamer *C17*), 95.2, 94.6 (major and minor rotamer *C*(CH_3)$_2$), 81.3, 80.6 (major and minor rotamer *C*(CH_3)$_3$), 67.3, 67.0 (major and minor rotamer *C29*-H_2), 62.7 (major and minor rotamer *C32*-H_2), 54.9, 54.8 (major and minor rotamer *C8*-H), 52.4, 52.3, 52.0 (major and minor rotamer *C8*-H, OCH_3), 28.3, 28.2 (major and minor rotamer C(*C*H_3)$_3$), 26.4, 25.4, 25.0, 24.2 (major and minor rotamer C(*C*H_3)$_2$), 13.8, 13.7 (major and minor rotamer C30-*C*H_3), 12.1 (major and minor rotamer C30-*C*H_3); *m/z* (ESI+) 547.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{23}H_{32}N_4O_8SNa$: 547.1833, found 547.1837 $[M+Na]^+$.

Trisazole 4.41

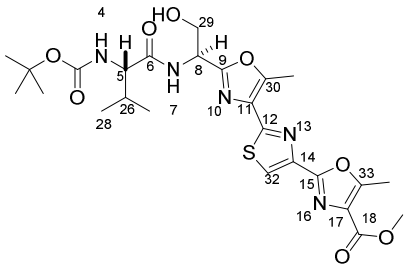


A solution of thioamide **4.40** (0.46 g, 0.88 mmol) in dry CH_2Cl_2 (8 mL) was cooled to -78 °C under a nitrogen atmosphere. (Diethylamino)sulfur trifluoride (0.21 mL, 1.6 mmol) was added dropwise and the reaction mixture was stirred at -78 °C for 1 hour, then to room temperature over 30 minutes. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (20 mL) and stirred for 15 minutes. The biphasic mixture was separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 5 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo* to give the crude thiazoline as a brown oil. This was used without further purification.

The solution of the crude thiazoline (0.88 mmol) in acetonitrile (2.9 mL), CCl_4 (2.0 mL), pyridine (2.9 mL) was cooled to 0 °C under a nitrogen atmosphere and 1, 8-Diazabicyclo[5.4.0]undec-7-ene (0.50 mL, 3.36 mmol) was added dropwise. The reaction was allowed to reach room temperature overnight. The reaction mixture was concentrated *in vacuo* and the residue was partitioned between pH 2 buffer (5 mL) and CH_2Cl_2 (5 mL), and the phases separated. The aqueous phase was further extracted with CH_2Cl_2 (3 x 5 mL), and the combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo* to give the crude thiazole. This was purified by silica chromatography (50 % EtOAc: pet. ether) to give the trisazole **4.41** as a clear yellow oil (0.27 g, 67 mmol, 77 %; $[\alpha]_D^{27}$ -112.5 (c = 0.11, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3373 (NH), 1689 (ester C=O), 1524 (NH); δ_H (600 MHz, $CDCl_3$) 8.07 (1H, s, (major rotamer C32-*H*), 8.05 (1H, s, minor rotamer C32-*H*), 5.14 (1H, br. s., minor rotamer

C8-*H*), 5.02 (1H, dd, *J* 6.0, 3.0 Hz, major rotamer C8-*H*), 4.31 - 4.21 (1H, m, major and minor rotamer C29-H_2), 4.16 (1H, dd, *J* 10.0, 3.0 Hz, major and minor rotamer C29-H_2), 3.95 (3H, s, major and minor rotamer CO_2CH_3), 2.81 (3H, m, major rotamer C30-CH_3), 2.78 (3H, m, minor rotamer C30-CH_3), 2.75 (3H, s, major and minor rotamer C33-CH_3), 1.78 (3H, s, major rotamer $C(CH_3)_2$), 1.75 (3H, br. s., minor rotamer $C(CH_3)_2$), 1.62 (3H, s, major rotamer $C(CH_3)_2$), 1.58 (3H, br. s., minor rotamer $C(CH_3)_2$), 1.51 (9H, s, minor rotamer $C(CH_3)_3$), 1.31 (9H, s, major rotamer $C(CH_3)_3$); δ_C (150 MHz, $CDCl_3$) 162.7, 162.4 (major and minor rotamer *C12*, *C18*), 161.5, 161.2 (major and minor rotamer *C9*), 156.5 (major and minor rotamer *C33*), (major and minor rotamer *C15*), 155.3 (major and minor rotamer *C15*), 152.0, 151.2 (major and minor rotamer $CO_2{}^tBu$), 148.0, 147.8 (major and minor rotamer *C30*), 143.6, 143.5 (major and minor rotamer *C14*), 129.8 (major and minor rotamer *C11*), 128.4 (major and minor rotamer *C17*), 120.3 (major and minor rotamer *C32*-H), 95.1, 94.6 (major and minor rotamer $C(CH_3)_2$), 81.1, 80.5 (major and minor rotamer $C(CH_3)_3$), 67.4, 67.2 (major and minor rotamer *C29*-H_2), 55.1, 55.0 (major and minor rotamer *C8*-H), 52.3, 52.1 (major and minor rotamer $COCH_3$), 28.3, 28.2 (major and minor rotamer $C(CH_3)_3$), 26.3, 25.2, 25.1, 24.3 (major and minor rotamer $C(CH_3)_2$), 12.2, 12.0 (major and minor rotamer C30-CH_3, C33-CH_3); *m/z* (ESI+) 527.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{23}H_{28}N_4O_7SNa$: 527.1571, found 527.1577 $[M+Na]^+$.

Peptapeptide 4.43



The protected trisazole **4.41** (0.24 g, 0.47 mmol) was dissolved in a solution of methanolic hydrochloric acid solution (4 M, 2.8 mL) and stirred at room temperature

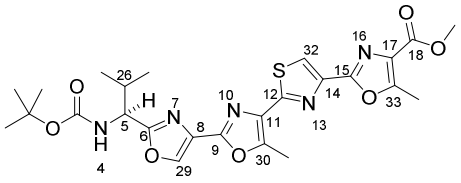
for 18 hours. The reaction mixture was concentrated *in vacuo* to give the crude deprotected amine as the hydrochloride salt, **4.42**, which was used without further purification, assuming quantitative yield.

A solution of Boc-Val-OH (0.15 g, 0.71 mmol) and HOBt (88 %, 0.017g, 0.11 mmol) in EtOH (1.5 mL) were stirred at room temperature for 15 minutes. To this was added a solution of the crude amine (0.47 mmol) and *N*-methylmorpholine (0.05 mL, 0.47 mmol) in EtOH (1.5 mL) and the reaction mixture was cooled to 0-5 °C. *N*-Methylmorpholine (0.20 mL, 1.8 mmol) was added and the reaction was stirred at 0-5 °C for 15 minutes. EDCI (0.16 g, 0.85 mmol) was added, and the reaction mixture allowed to reach room temperature overnight. The reaction mixture was acidified with pH 2 buffer, and concentrated *in vacuo*. The residue was partitioned between pH 2 buffer (5 mL) and CH_2Cl_2 (10 mL) and the phases were separated. The aqueous phase was further extracted with CH_2Cl_2 (3 x 5 mL) and the combined organic extracts were washed with water (5 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The pentapeptide **4.43** was obtained by silica chromatography (5 - 10 % MeOH: CH_2Cl_2) as a white crystalline solid (0.11 g, 0.20 mmol, 42 %); m.p. 221 - 224; $[\alpha]_D^{25}$ -17.9 (c = 0.32, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3314 (NH), 1722 (ester C=O), 1650 (amide C=O), 1524 (NH); δ_H (400 MHz, $CDCl_3$) 8.06 (1H, s, C32-*H*), 7.10 (1H, d, *J* 8.0 Hz, C7-*H*), 5.30 (1H, dt, *J* 8.0, 4.0 Hz, *C8*-H), 5.20 (1H, d, *J* 8.0 Hz, N4-*H*), 4.23 - 3.97(3H, m, C5-*H*, C29-H_2), 3.95 (3H, s, OCH_3), 2.77 (3H, s, C30-CH_3), 2.74 (3H, s, C30-CH_3), 2.23 - 2.08 (1H, m, C26-*H*), 1.46 - 1.41 (9H, m, C(CH_3)$_3$), 1.04 - 0.93 (6H, m, C28-H_3); δ_C (150 MHz, $CDCl_3$) 171.9 (*C6*), 162.7, 161.7, 159.9 (*C9*, *C12*, *C18*), 156.5 (*C33*), 155.3, *C15*), 152.8 (*C*O_2^{t}Bu), 148.7 (*C30*), 143.6 (*C14*), 129.7 (*C11*), 128.4 (*C17*), 120.2 (*C32*-H), 81.0 (*C*$(CH_3)_3$), 63.2 (*C29*-H_2), 60.4 (*C5*-H), 52.1 (O*C*H_3), 49.2 (*C*8-*H*), 30.8 (*C26*-H), 28.3 (C(*C*H_3)$_3$), 19.2, 18.0 (*C28*-H_3), 12.2, 12.0 (C30-*C*H_3, C33-*C*H_3); *m/z* (ESI+) 586.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{25}H_{33}N_5O_8SNa$: 586.1942, found 586.1931 $[M+Na]^+$.

Preparation of activated MnO_2, Method 1

Prepared according to the method of Carpino.[30] A solution of $KMnO_4$ (12.0 g, 75 mmol) in water (150 mL) in a 500 mL beaker was heating to boiling point with stirring. The solution was removed from the heat, and activated carbon (7.5 g) was added over 10 minutes. The mixture was heated to boiling point until no purple colour was observable (ca. 5 minutes). After cooling for 15 minutes, the reaction mixture was filtered, and the filter cake washed with water (4 x 25 mL), until the washes were colourless. The filtercake was dried under vacuum for 5 minutes, suspended in toluene (75 mL) and residual water was removed by azeotrope using a Dean-Stark apparatus. The suspension was cooled, filtered and the filtercake dried *in vacuo* to give carbon supported MnO_2 as black powdery solid (8.0 g).

Pentapeptide 4.35

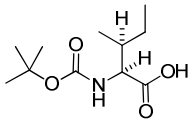


(Diethylamino)sulfur trifluoride (0.05 mL, 0.36 mmol) was added to a solution of pentapeptide **4.43** (0.10 g, 0.18 mmol) in dry CH_2Cl_2 (2 mL) at -78 °C under an atmosphere of nitrogen. The reaction mixture was stirred at -78 °C for 3.5 hours, and then at room temperature for 30 minutes. The mixture was quenched with saturated aqueous $NaHCO_3$ solution (2 mL) and was stirred for 15 minutes. The phases were separated and the aqueous phase was further extracted with CH_2Cl_2 (5 x 2 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. The oxazoline was obtained by silica chromatography (50 % EtOAc: pet ether) as a white solid (69 mg, 0.13 mmol, 70 %) and was used immediately.

Activated MnO_2 (prepared according to Method 1, 0.25 g) was added to a solution of the oxaozline (50 mg, 0.09 mmol) in dry toluene (2.5 mL) under a nitrogen atmosphere. The reaction mixture was heated to reflux for 13 hours with azeotropic

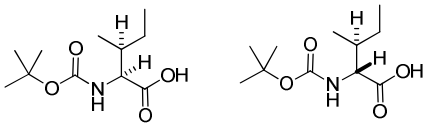
removal of water over 3Å molecular sieves. Additional MnO_2 (0.3 g) and dry toluene (5 mL) were added after 3 hours. The reaction mixture was cooled to room temperature and filtered through celite. The celite was washed with EtOAc (5 x 5 mL). The filtrate was concentrated *in vacuo* and purified by silica chromatography to give the oxazole 4.35 as a bright white solid (8 mg, 15 µmol, 16 % from oxazoline, 4 % over 2 steps). The characterization data were identical to that prepared in the previous method.

_N-tert_Butoxycarbonyl-L-isoleucine 4.45



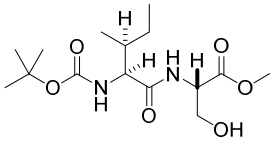
Prepared as for Boc-Val-OH, **4.19,** but using L-isoleucine (10.0 g, 76.2 mmol). The Boc-protected product, **4.45**, was obtained as a clear, colourless oil (14.5 g, 62.5 mmol, 82 %); $[\alpha]_D^{25}$ + 3.80 (c =1.01, MeOH); (lit.[31] $[\alpha]_D^{20}$+3.9 (c = 2.00, MeOH); υ_{max}/cm^{-1} (neat) 3321 (NH), 2967 (OH), 1708 (acid C=O), 1503 (NH); δ_H (400 MHz, $CDCl_3$) 10.36 (1H, br. s., major and minor rotamer CO_2H), 6.16 (1H, br. s, minor rotamer CHN*H*), 5.13 (1H, d, *J* 8.5 Hz, major rotamer CHN*H*), 4.25 (1H, dd, *J* 8.5, 4.5 Hz, major rotamer C*H*NH), 4.12 – 4.06 (1H, m, major rotamer C*H*NH), 1.99 - 1.81 (1H, m, C*H*CH_3), 1.53 - 1.42 (10H, m, C(C*H*$_3$)$_3$, C*H*$_2$$CH_3$), 1.28 – 1.08 (1H, m, C*H*$_2$$CH_3$), 0.96 (3H, d, *J* 6.5 Hz, CHC*H*$_3$), 0.92 (3H, t, *J* 7.5 Hz, CH_2C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 177.2 (*C*O_2H), 155.8 (*C*O_2^{t}Bu), 80.0 (*C*$(CH_3)_3$), 58.1 (*C*HNH), 37.7 (*C*HCH_3), 28.3 (C(*C*H$_3$)$_3$), 24.9 (*C*H_2CH_3), 15.5 (CH*C*H_3), 11.6 (CH_2*C*H_3); *m/z* (ESI+) 230.1 ([M-H] 100%); HR-ESIMS: calculated for $C_{11}H_{21}NO_4Na$: 254.1363, found 254.1350 $[M+Na]^+$. The data are consistent with that previously reported.[31]

_N-tert_Butoxycarbonyl-L-isoleucine and _N-tert_Butoxycarbonyl-D-_allo_-isoleucine 4.46



Prepared as for Boc-Val-OH, **4.19**, but using a mixture of L-isoleucine and D-*allo*-isoleucine **3.11** (0.5 g, 3.81 mmol). The Boc-protected product **4.46** was obtained as a clear, colourless oil (0.77 g, 3.34 mmol, 88 %, 73: 27 mix of (2*S*, 3*S*) and (2*R*, 3*S*), calculated by ^{1}H NMR); υ_{max}/cm^{-1} (neat) 3314 (NH), 2966 (OH), 1711 (acid C=O), 1506 (NH); δ_H (400 MHz, $CDCl_3$) 8.71 ((2*S,* 3*S*), 1H, br. s., major and minor rotamer CO_2*H*; (2*R,* 3*S*), 1H, br. s., major and minor rotamer CO_2*H*), 6.17 ((2*S,* 3*S*), 1H, d, *J* 7.0 Hz, minor rotamer CHN*H*), 6.08 ((2*R,* 3*S*), 1H, d, *J* 7.0 Hz, minor rotamer CHN*H*), 5.06 ((2*S,* 3*S*), 1H, d, *J* 8.5 Hz, major rotamer CHN*H*), 5.00 ((2*R,* 3*S*), 1H, d, *J* 9.0 Hz, major rotamer CHN*H*), 4.41 ((2*R,* 3*S*), 1H, dd, *J* 9.5, 4.0 Hz, major rotamer C*H*NH), 4.30 ((2*S,* 3*S*), 1H, dd, *J* 9.0, 4.5 Hz, major rotamer C*H*NH), 4.24 - 4.17 ((2*R,* 3*S*), 1H, m, minor rotamer C*H*NH), 4.13 - 4.05 ((2*S,* 3*S*), 1H, m, minor rotamer C*H*NH), 2.03 - 1.83 ((2*S,* 3*S*), 1H, m, major and minor rotamer C*H*CH_3; (2*R,* 3*S*), 1H, m, major and minor rotamer C*H*CH_3), 1.45 ((2*S,* 3*S*), 10H, m, major and minor rotamer C*H*$_2CH_3$, C(C*H*$_3$)$_3$; (2*R,* 3*S*), 10H, m, major and minor rotamer C*H*$_2CH_3$, C(C*H*$_3$)$_3$), 1.31 - 1.14 ((2*S,* 3*S*), 1H, m, major and minor rotamer C*H*$_2CH_3$; (2*R,* 3*S*), 1H, m, major and minor rotamer C*H*$_2CH_3$), 0.99 - 0.87 ((2*S,* 3*S*), 6H, m, major and minor rotamer CH_2CH_3, $CHCH_3$; (2*R,* 3*S*), 1H, m, major and minor rotamer CH_2CH_3, $CHCH_3$); δ_C (100 MHz, $CDCl_3$) 177.8, 177.3 ((2*S,* 3*S*), *C*O_2H; (2*R,* 3*S*), *C*O_2H), 155.8 ((2*S,* 3*S*), *C*O_2 ^{t}Bu; (2*R,* 3*S*), *C*O_2^{t}Bu), 80.1 ((2*S,* 3*S*), *C*$(CH_3)_3$; (2*R,* 3*S*), *C*$(CH_3)_3$), 59.1, 57.8 ((2*S,* 3*S*), *C*HNH; (2*R,* 3*S*), *C*HNH), 37.7, 37.4 ((2*S,* 3*S*), *C*HCH_3; (2*R,* 3*S*), *C*HCH_3), 28.3 ((2*S,* 3*S*), C(*C*H_3)$_3$; (2*R,* 3*S*), C(*C*H_3)$_3$), 26.3, 24.9 ((2*S,* 3*S*), *C*H_2CH_3; (2*R,* 3*S*), *C*H_2CH_3), 15.5 ((2*S,* 3*S*), CH*C*H_3; (2*R,* 3*S*), CH*C*H_3), 11.7 ((2*S,* 3*S*), CH_2*C*H_3; (2*R,* 3*S*), CH_2*C*H_3); *m/z* (ESI+) 230.1 ([M+H] 100%); HR-ESIMS: calculated for $C_{11}H_{20}NO_4$: 230.1398, found 230.1385 $[M+H]^+$.

***N-tert*Butoxycarbonyl-L-isoleucyl-L-serine methyl ester 4.47**

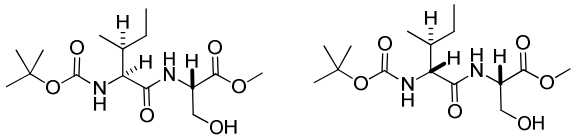


Acetyl chloride (5.2 mL, 73.2 mmol) was added dropwise with stirring to MeOH (30 mL) at 0 °C. The mixture was allowed to reach room temperature and L-serine (1.30 g, 12.2 mmol) was added and the mixture was heated to reflux for 18 hours. The reaction mixture was concentrated *in vacuo* to give the crude methyl ester as the hydrochloride salt, which was used without further purification assuming quantitative yield.

A solution of Boc-L-isoleucine **4.45** (0.93 g, 4.0 mmol) and HOBt (88 %, 0.09 g, 0.60 mmol) in EtOH (13 mL) was stirred at room temperature for 15 minutes, then cooled to 0-5°C. To this was added a solution of the crude serine methyl ester (4.42 mmol) and *N*-methyl morpholine (1.4 mL, 12.9 mmol) in EtOH (9 mL) and the resulting colourless solution stirred at 0-5°C for 15 minutes. EDCI (0.92 g, 4.8 mmol) was added and the reaction mixture was allowed to reach room temperature overnight to give a pale yellow solution. This was acidified using pH 2 buffer and *N*-methyl morpholine hydrosulfate was removed by filtration. The filtrate was concentrated *in vacuo*, and extracted with EtOAc (4 x 10 mL). The combined organic extracts were washed with pH 2 buffer (10 mL), water (10 mL), saturated aqueous $NaHCO_3$ solution (10 mL) and saturated aqueous NaCl solution (10 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give dipeptide **4.47** as a white powdery solid (0.79 g, 2.39 mmol, 60 %); m.p. 83 – 85 °C; $[\alpha]_D^{25}$ -19.1 (c = 0.26, MeOH); υ_{max}/cm^{-1} (neat) 3323 (NH), 1748 (ester C=O), 1688 (amide C=O), 1520 (NH); δ_H (400 MHz, $CDCl_3$) 7.20 (1H, d, *J* 7.5 Hz, Ser N*H*CH), 5.38 (1H, d, *J* 8.0 Hz, Ile N*H*CH), 4.67 (1H, ddd, *J* 8.0, 4.0, 3.0 Hz, Ser NHC*H*), 4.01 - 3.85 (3H, m,

Ile NHC*H*, CH_2OH), 3.76 (3H, s, OCH_3), 1.88 - 1.74 (1H, m, C*H*CH$_3$), 1.61 - 1.50 (1H, m, CH_2CH$_3$), 1.42 (9H, s, C(CH_3)$_3$), 1.21 - 1.08 (1H, m, CH_2CH$_3$), 0.95 (3H, d, *J* 6.5 Hz, C*H*CH$_3$), 0.89 (3H, t, *J* 7.5 Hz, CH$_2$CH_3); δ_C (100 MHz, $CDCl_3$) 172.3 (*C*O$_2$CH$_3$), 170.8 (*C*ONH), 156.3 (*C*O$_2$^{t}Bu), 80.2 (*C*(CH$_3$)$_3$), 62.7 (*C*H$_2$OH), 59.4 (Ile *C*HNH), 57.9 (Ser *C*HNH), 52.6 (O*C*H$_3$), 37.2 (*C*HCH$_3$), 28.3 (C(*C*H$_3$)$_3$), 24.8 (*C*H$_2$CH$_3$), 15.4 (CH*C*H$_3$), 11.3 (CH$_2$*C*H$_3$); *m/z* (ESI+) 355.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{15}H_{28}N_2O_6Na$: 355.1840, found 355.1823 $[M+Na]^+$. The ^{1}H and ^{13}C sprectoscopic data are consistent to that previously reported.[32]

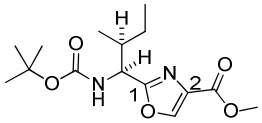
_N-tert_Butoxycarbonyl-L-isoleucyl- and _N-tert_Butoxycarbonyl-D-_allo_-isoleucyl-L-serine methyl ester 4.48



Prepared as for the single diastereoisomer using L-serine methyl ester hydrochloride (3.44 mmol) and a mixture of L-isoleucine and D-*allo*-isoleucine, **3.12** (0.72 g, 3.12 mmol), *N*-methyl morpholine (1.0 mL, 10 mmol), HOBt (88 %, 0.07 g, 0.5 mmol), EDCI (0.72 g, 3.8 mmol) and EtOH (17 mL) to give the dipeptide as a bright white solid (0.79 g, 2.37 mmol, 76 %; 78: 22 mix of (2*S,* 3*S*) and (2*R*, 3*S*) diastereomers by ^{1}H NMR); υ_{max}/cm^{-1} (neat) 3322 (NH), 1748 (ester C=O), 1689 (amide C=O), 1522 (NH); δ_H (400 MHz, $CDCl_3$) 7.26 (1H, d, *J* 8.0 Hz, (2*S,* 3*S*), Ser CHN*H*; 1H, d, *J* 8.0 Hz, (2*R,* 3*S*), Ser CHN*H*), 5.41 (1H, d, *J* 9.0 Hz, (2*S,* 3*S*), Ile CHN*H*), 5.20 (1H, d, *J* 7.5 Hz, (2*R,* 3*S*), Ile CHN*H*), 4.66 (1H, dt, *J* 7.5, 3.5 Hz, (2*S,* 3*S*), Ser C*H*NH; 1H, dt, *J* 7.5, 3.5 Hz, (2*R,* 3*S*), Ser C*H*NH), 4.19 - 4.11 (1H, m, (2*R,* 3*S*), Ile C*H*NH), 3.98 (1H, dd, *J* 8.5, 7.5 Hz, (2*S,* 3*S*), Ile C*H*NH), 3.98 – 3.83 (2H, m, (2*S,* 3*S*), CH_2OH; 2H, m, (2*R,* 3*S* CH_2OH), 3.75 (3H, m, (2*S,* 3*S*), OCH_3; 3H, m, (2*R,* 3*S*), OCH_3), 2.06 - 1.94 (1H, m, (2*R,* 3*S*), C*H*CH$_3$), 1.86 – 1.74 (1H, m, (2*S,* 3*S*), C*H*CH$_3$), 1.65 - 1.48 (1H, m, (2*S,* 3*S*), CH_2CH$_3$; 1H, m, (2*R,* 3*S*), CH_2CH$_3$), 1.42 (9H, s, (2*R,* 3*S*), C(CH_3)$_3$), 1.40 (9H, s, (2*S,* 3*S*), C(CH_3)$_3$), 1.29 – 1.07 (1H, m, (2*S,* 3*S*), CH_2CH$_3$; 1H, m, (2*S,* 3*S*), CH_2CH$_3$), 0.97 - 0.85 (1H, m, (2*S,* 3*S*),

CHC*H*$_3$,CH$_2$C*H*$_3$; 1H, m, (2*S,* 3*S*), CHC*H*$_3$, CH$_2$C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 172.3 ((2*S,* 3*S*), *C*O$_2$H; (2*R,* 3*S*), *C*O$_2$H), 171.0, 170.8 ((2*S,* 3*S*), *C*ONH; (2*R,* 3*S*), *C*ONH), 156.3 ((2*S,* 3*S*), *C*O$_2$^{t}Bu; (2*R,* 3*S*), *C*O$_2$^{t}Bu), 80.4, 80.2 ((2*S,* 3*S*), *C*(CH$_3$)$_3$; (2*R,* 3*S*), *C*(CH$_3$)$_3$), 62.6 ((2*S,* 3*S*), *C*H$_2$OH; (2*R,* 3*S*), *C*H$_2$OH), 59.4, 58.4 ((2*S,* 3*S*), Ile NH*C*H; (2*R,* 3*S*), Ile NH*C*H), 54.7 ((2*S,* 3*S*), Ser NH*C*H; (2*R,* 3*S*), Ser NH*C*H), 52.7, 52.6 ((2*S,* 3*S*), O*C*H$_3$; (2*R,* 3*S*), O*C*H$_3$), 37.3, 37.0 ((2*S,* 3*S*), *C*HCH$_3$; (2*R,* 3*S*), *C*HCH$_3$), 28.3 ((2*S,* 3*S*), C(*C*H$_3$)$_3$; (2*R,* 3*S*), C(*C*H$_3$)$_3$), 24.8 ((2*S,* 3*S*), *C*H$_2$CH$_3$; (2*R,* 3*S*), *C*H$_2$CH$_3$), 15.4, 14.2 ((2*S,* 3*S*), CH*C*H$_3$; (2*R,* 3*S*), CH*C*H$_3$), 11.7, 11.2 ((2*S,* 3*S*), CH$_2$*C*H$_3$; (2*R,* 3*S*), CH$_2$*C*H$_3$); *m/z* (ESI+) 355.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{15}H_{28}N_2O_6Na$: 355.1840, found 355.1839 $[M+Na]^+$.

(2*S*, 3*S*) Oxazole 4.49

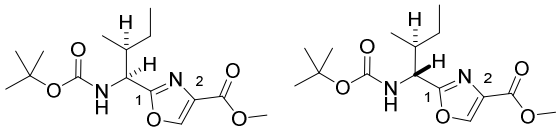


(Diethylamino)sulfur trifluoride (0.17 mL, 1.3 mmol) was added to solution of the dipeptide **4.47** (0.31 g, 0.92 mmol) in dry CH_2Cl_2 (8.5 mL) at -78 °C under a nitrogen atmosphere. The reaction mixture was stirred at -78 °C for 1.5 hours, and at room temperature for 30 minutes. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (10 mL) and stirred for 15 minutes. The phases were separated and the aqueous phase was further extracted with CH_2Cl_2 (2 x 5 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo* to give the crude oxazoline as a yellow oil that was used immediately assuming quantitative yield.

MnO_2 (prepared by the method of Carpino,[30] *vide supra*, 5 x mass oxazoline, 1.25 g) was added to a solution of the oxazoline (0.25 g, 0.92 mmol) in dry toluene (12 mL) under a nitrogen atmosphere. The reaction was heated to reflux with azeotropic removal of water over 3Å molecular sieves for 20 hours. After cooling to room temperature, the mixture was filtered through celite. The celite was washed with EtOAc (5 x 5 mL) and the filtrate was concentrated *in vacuo*. The oxazole was

obtained by silica chromatography (50% EtOAc: pet. ether) as a white solid (7 mg, 0.02 mmol, 2 %); $[\alpha]_D^{25}$ -19.3 (c = 0.64, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3326 (NH), 2972 (aromatic C-H), 1727 (ester C=O), 1698 (amide C=O), 1529 (NH); δ_H (400 MHz, $CDCl_3$) 8.18 (1H, s, oxazole C-*H*), 5.33 (1H, d, *J* 9.0 Hz, N*H*CH), 4.86 (1H, dd, *J* 9.0, 6.0 Hz, NHC*H*), 3.91 (3H, s, OCH_3), 2.01 - 1.88 (1H, m, C*H*CH_3), 1.51 – 1.39 (10H, m, CH_2CH_3, C(CH_3)$_3$), 1.24 – 1.10 (1H, m, CH_2CH_3), 0.90 (3H, t, *J* 7.0 Hz, CH_2CH_3), 0.86 (3H, d, *J* 7.0 Hz, CHCH_3); δ_C (100 MHz, $CDCl_3$) 164.1 (*C1*), 160.6 (*C*O_2Me), 154.2 (*C*$O_2{}^t$Bu), 142.8 (oxazole C-*H*), 132.1 (*C2*), 79.0 (*C*$(CH_3)_3$), 52.3 (*C*HNH), 51.2 (O*C*H_3), 38.5 (*C*HCH_3), 28.7 (C(*C*H_3)$_3$), 24.0 (*C*H_2CH_3), 14.3 (CH*C*H_3),10.4 (CH_2*C*H_3); *m/z* (ESI+) 335.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{15}H_{24}N_2O_5Na$: 335.1577, found 335.1572 $[M+Na]^+$. This compound is known, but no characterisation data has been reported.[33]

(2*S*, 3*S*) and (2*R*, 3*S*) Oxazole 4.50



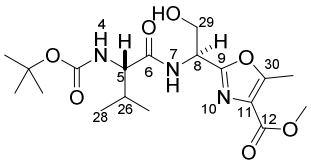
Prepared as for the single diastereoisomer, **4.49**, using a diastereomeric mix of dipeptide **4.48** (0.39 g, 1.17 mmol), (diethylamino)sulfur trifluoride (0.22 mL, 1.6 mmol) and CH_2Cl_2 (11 mL) to give the oxazoline. The oxazole **4.50** was prepared as for the single diastereomer with MnO_2 (2.2 g) and dry toluene (10 mL). Additional MnO_2 (0.85 g) and dry toluene (5 mL) were added after 2 hours at reflux, and the reaction mixture was heated to reflux for a further 7 hours. The cooled reaction mixture was filtered over celite, and the celite washed with EtOAc (5 x 10 mL). The filtrate was concentrated *in vacuo* and the oxazole, **4.50**, obtained by silica chromatography (50% EtOAc: pet. ether) as a white solid (3 mg, 0.09 mmol, 1 %; 72: 28 mix of (2*S*, 3*S*) and (2*R*, 3*S*) diastereomers by ^{1}H NMR): δ_H (400 MHz, $CDCl_3$) 8.18 (1H, s, (2*S,* 3*S* CC-*H*; 1H, s, (2*R,* 3*S*), CC-*H*), 5.32 (1H, d, *J* 9.0 Hz, (2*S,* 3*S*), N*H*CH), 5.27 (1H, d, *J* 9.5 Hz, (2*S,* 3*S*), N*H*CH), 4.95 (1H, dd, *J* 9.5 Hz,

5.0 Hz, (2*R,* 3*S*), NHC*H*), 4.86 (1H, dd, *J* 9.0 Hz, 6.5 Hz, (2*S,* 3*S*), NHC*H*), 3.91 (3H, s, (2*S,* 3*S*), OCH_3; 3H, s, (2*R,* 3*S*), OCH_3), 2.02 – 1.88 (1H, m, (2*S,* 3*S*), C*H*CH_3; 1H, m, (2*R,* 3*S*), C*H*CH_3), 1.50 – 1.39 (10H, m, (2*S,* 3*S*), C$H_2$$CH_3$, C(C$H_3$)$_3$; 10H, m, (2*R,* 3*S*), C$H_2$$CH_3$, C(C$H_3$)$_3$), 1.23 – 1.10 (1H, d, *J* 7.0 Hz, (2*S,* 3*S*), C$H_2$$CH_3$; 1H, d, *J* 7.0 Hz, (2*R,* 3*S*), C$H_2$$CH_3$), 0.96 - 0.83 (6H, m, (2*S,* 3*S*), CHCH_3, CH_2CH_3; 6H, m, (2*R,* 3*S*), CHCH_3, CH_2CH_3); δ_C (100 MHz, $CDCl_3$) 165.2 ((2*S,* 3*S*), *C1*; (2*R,* 3*S*), *C1*), 161.6 ((2*S,* 3*S*), *C*O_2Me; (2*R,* 3*S*), *C*O_2Me), 155.3 ((2*S,* 3*S*), *C*O_2^{t}Bu; (2*R,* 3*S*), *C*O_2^{t}Bu), 143.8 ((2*S,* 3*S*), C*C*-H; (2*R,* 3*S*), C*C*-H), 133.2 ((2*S,* 3*S*), *C2*; (2*R,* 3*S*), *C2*), 80.1 ((2*S,* 3*S*), *C*$(CH_3)_3$; (2*R,* 3*S*), *C*$(CH_3)_3$), 53.4, 52.7 ((2*S,* 3*S*), *C*HNH; (2*R,* 3*S*), *C*HNH), 51.6 ((2*S,* 3*S*), O*C*H_3; (2*R,* 3*S*), O*C*H_3), 39.5, 39.3 ((2*S,* 3*S*), *C*HCH_3; (2*R,* 3*S*), *C*HCH_3), 28.3 ((2*S,* 3*S*), C(*C*H_3)$_3$; (2*R,* 3*S*), C(*C*H_3)$_3$), 25.9, 25.1 ((2*S,* 3*S*), *C*$H_2$$CH_3$); (2*R,* 3*S*), *C*$H_2$$CH_3$), 15.2, 14.5 (2*S,* 3*S*), CH*C*H_3; (2*R,* 3*S*), CH*C*CH_3), 11.6, 11.4 ((2*S,* 3*S*), CH_2*C*H_3; (2*R,* 3*S*), CH_2*C*H_3); *m/z* (ESI+) 335.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{15}H_{24}N_2O_5Na$: 335.1577, found 335.1582 $[M+Na]^+$.

Preparation of activated MnO_2 (Method 2):

Prepared according to the method set out by Fatiadi.[34] A solution of $KMnO_4$ (26.3 g, 167 mmol) in water (500 mL) was added slowly to a solution of manganese sulfate monohydrate (42.3 g, 250 mmol) in water (720 mL) at 60 ˚C in a 2L beaker. The solution was stirred at 60 °C for 1 hour. After cooling to room temperature, the suspension was filtered. The filtercake was washed with water (~2L) until the pH of the aqueous washes was neutral. The filtercake was dried under vacuum over silica for to give a dense black solid (86.1 g), to give the unactivated MnO_2 with ~68% associated water. This was activated immediately before use according to the method of Goldman.[35]

Tripeptide Oxazole 4.51

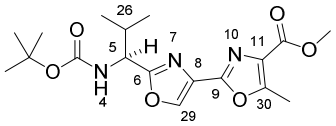


Oxazole methyl ester **4.10** (3.05 g, 8.96 mmol) was dissolved in a solution of methanolic hydrochloric acid (4 M, 21.5 mL) and stirred at room temperature for 18 hours. The reaction mixture was concentrated *in vacuo* to give the crude deprotected amine as the hydrochloride salt, which was used without further purification assuming quantitative yield.

A solution of Boc-Val-OH (2.83 g, 13.4 mmol) and HOBt (88 %, 0.32g, 2.06 mmol) in EtOH (50 mL) were stirred at room temperature for 15 minutes. To this was added a solution of the crude amine (8.96 mmol) in EtOH (40 mL) and the reaction mixture was cooled to 0-5 °C. *N*-Methylmorpholine (4.7 mL, 43.0 mmol) was added and the reaction was stirred at 0-5 °C for 15 minutes. EDCI (3.08 g, 16.1 mmol) was added, and the reaction mixture allowed to reach room temperature overnight. The reaction mixture was acidified with pH 2 buffer, filtered, and the filtrate was concentrated *in vacuo*. The residue was partitioned between pH 2 buffer (50 mL) and EtOAc (100 mL) and the phases were separated. The organic phase was washed with pH 2 buffer (25 mL), water (25 mL), saturated aqueous $NaHCO_3$ solution (25 mL) and saturated aqueous NaCl solution (25 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The pure tripeptide, **4.51**, was obtained by silica chromatography (EtOAc) as a bright white crystalline solid (2.05 g, 5.13 mmol, 57 %; also performed with 1.68 g, 88 %); m.p. 99 – 100 °C; $[\alpha]_D^{25}$ -47.3 (c = 0.52, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3326 (NH), 3306 (OH), 1721 (ester C=O), 1687 (amide C=O), 1519 (NH);^{1}H NMR (400 MHz, $CDCl_3$) 7.50 (1H, d, *J* 8.0 Hz, N7-*H*), 5.43 (1H, d, *J* 8.5 Hz, N4-*H*), 5.22 (1H, dt, *J* 8.0, 4.0 Hz, C8-*H*), 4.23 - 4.16 (1H, m, CH_2OH), 4.02 – 3.96 (2H, m, C5-*H*, CH_2OH), 3.95 – 3.86 (1H, m, CH_2OH), 3.80 (3H, s, OCH_3), 2.51 (3H, s, oxazole

C*H*$_3$), 2.11 - 2.00 (1H, m, C*H*CH$_3$), 1.35 (9H, s, C(C*H*$_3$)$_3$), 0.90 (3H, d, *J* 7.0 Hz, CH(C*H*$_3$)$_2$), 0.86 (3H, d, *J* 6.5 Hz, CH(C*H*$_3$)$_2$); δ_C (175 MHz, $CDCl_3$) 172.1 (*C6*), 162.4 (*C12*), 159.8 (*C9*), 156.9 (*C30*), 156.2 (*C*O$_2$^{t}Bu), 127.3 (*C11*), 80.2 (*C*(CH$_3$)$_3$), 63.1 (*C29*-H$_2$), 60.2 (*C5*-H), 52.0 (O*C*H$_3$), 49.3 (*C8*-H), 30.8 (*C26*-H), 28.3 (C(*C*H$_3$)$_3$), 19.2 (*C*28-H$_3$), 17.8 (*C*28-H$_3$), 12.0 (C30-*C*H$_3$); *m/z* (ESI+) 422.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{18}H_{30}N_3O_7$: 400.2078, found 400.2087 $[M+H]^+$.

Bisoxazole 4.35

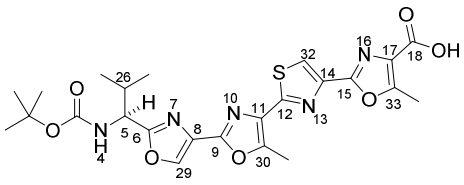


(Diethylamino)sulfur trifluoride (1.22 mL, 9.24 mmol) was added dropwise to a solution of the tripeptide **4.51** (2.05 g, 5.13 mmol) in dry CH_2Cl_2 (55 mL) at – 78 °C under a nitrogen atmosphere. The recation mixture was stirred at -78 °C for 2 hours, then at room temperature for 30 minutes. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (55 mL) and stirred for 15 minutes. The phases were separated and the organic phase was further extracted with CH_2Cl_2 (2 x 20 ml). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. The crude oxazoline was used immediately without further purification

A suspension of MnO_2 (prepared by the method of Fatiadi,[34] 17.8 g, 103 mmol) in toluene (100 mL) was heated to reflux with azeotropic water removal using Dean-Stark apparatus until no more water could be removed. A solution of the oxazoline (5.13 mmol) in toluene (10 mL) was added and the reaction heated to reflux with azeotrpic water removal for 6 hours. After cooling to room temperature, an acidic solution of sodium sulphite (1.5 g Na_2SO_3/1 g MnO_2 in 5 volumes of water, acidified to pH 5 with concentrated H_2SO_4) was added to the reaction mixture and the pH of the aqueous phase was adjusted to 5 and stirred until dissolution of the suspension. The phases were separated and the aqueous phase was acidified to pH 2 and

extracted with EtOAc (3 x 50 mL). The combined organic phases were washed with water (100 mL), saturated aqueous $NaHCO_3$ solution (100 mL), and saturated aqueous NaCl solution (100 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The bisoxazole **4.35** was obtained by silica chromatography (50 % EtOAc: pet. ether) as a bright white solid (0.53 g, 1.40 mmol, 27% over 2 steps); All characterisation data was identical to that obtained for the previous method.

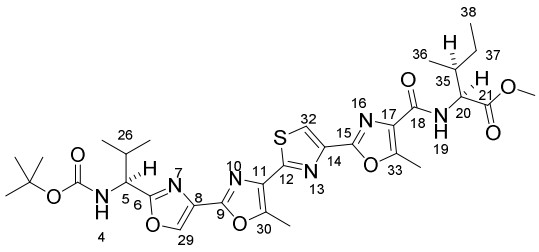
Tetrazole Carboxylic Acid 4.52



LiOH (0.08 g, 3.49 mmol) was added to a stirred suspension of methyl ester **4.35** (0.19 g, 0.35 mmol) in MeOH (15 mL), THF (15 mL) and water (2.7 mL). After 18 hours at room temperature, THF (5 mL), MeOH (5 mL) and CH_2Cl_2 (3 mL) were added to the suspension, and the mixture stirred for a further 8 hours, until complete by TLC (EtOAc). The mixture was acidified with pH 2 buffer, and the organics removed *in vacuo*. The mixture was extracted with CH_2Cl_2 (5 x 3 mL), and the combined organics were dried over Na_2SO_4 and concentrated *in vacuo* to give the acid **4.52** as a bright white solid (0.17 g, 0.32 mmol, 92%); m.p. 208 - 210 °C; $[\alpha]_D^{24}$ -53.8 (c = 0.25, MeOH); υ_{max}/cm^{-1} (neat) 3332 (NH), 3120 (OH), 1685 (acid C=O), 1620 (amide C=O), 1518 (NH); δ_H (600 MHz, $CDCl_3$) 8.23 (1H, s, C29-*H*), 8.11 (1H, s, C32-*H*), 5.86 (1H, br.s, minor rotamer N4-*H*), 5.40 (1H, d, *J* 9.5 Hz, major rotamer N4-*H*), 4.86 (1H, dd, J 9.0, 6.5 Hz, major rotamer C5-*H*), 4.70 (1H, br. s., minor rotamer C5-*H*), 2.88 (3H, s, C30-CH_3), 2.77 (3H, s, C33-CH_3), 2.35 - 2.18 (1H, m, C26-*H*), 1.54 - 1.37 (9H, m, C(CH_3)$_3$), 0.98 (3H, d, *J* 7.0 Hz, C26(CH_3)$_2$), 0.95 (3H, d, *J* 7.0 Hz, C26(CH_3)$_2$); δ_C (150 MHz, $CDCl_3$) 165.7 (*C6*), 165.2 (*C*O_2H), 162.2 (*C12*), 157.3 (*C33*), 155.4, 155.3 (*C*O_2^tBu, *C15*), 153.3 (*C30*), 143.4 (*C14*), 140.23 (*C29*-H), 130.8 (*C11*), 129.9 (*C8*), 128.2 (*C17*), 120.8 (*C32*-H), 80.1 (*C*$(CH_3)_3$), 54.4 (*C5*-H), 33.0 (*C26*), 28.3 (C(*C*H_3)$_3$), 18.8, 18.1 (C26(*C*H_3)$_2$), 12.3,

12.2 (C30-CH_3, C33-CH_3); *m/z* (ESI-) 528.0 ([M-H] 100%); HR-ESIMS: calculated for $C_{24}H_{27}N_5O_7SNa$: 552.1523, found 552.1540 $[M+Na]^+$.

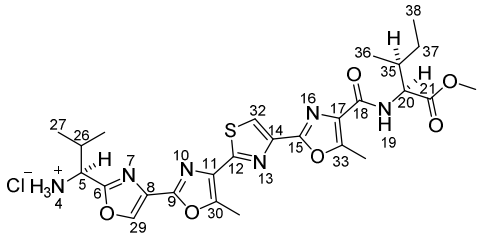
Hexapeptide 4.53



HOBt (88 %, 5 mg, 0.032 mmol) was added to a solution of carboxylic acid **4.52** (86 mg, 0.16 mmol) in EtOH (2 mL), and the reaction mixture was stirred for 15 minutes at room temperature. To this was added a solution of L-isoleucine methyl ester hydrochloride (70 mg, 0.40 mmol) in EtOH (2 mL) and the mixture was cooled to 0-5 °C. *N*-Methyl morpholine (0.11 mL, 1.05 mmol) was added and the mixture was stirred at 0-5 °C for 15 minutes. EDCI (46 mg, 0.24 mmol) was added and the reaction mixture allowed to reach room temperature overnight. The resulting dense white suspension was acidified with pH 2 buffer, and extracted with EtOAc (5 x 5 mL). The combined organics were washed with saturated aqueous NaCl solution (5 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the crude hexapeptide. Hexapeptide **4.53** was obtained by silica chromatography (2% MeOH: CH_2Cl_2) as a pale yellow crystalline solid (89 mg, 0.136 mmol, 84 %); m.p. 127 - 129 °C; $[\alpha]_D^{24}$ -18.4 (c = 0.51, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3305 (NH), 1742 (ester C=O), 1680, 1630 (amide C=O), 1510 (NH); δ_H (500 MHz, $CDCl_3$) 8.23 (1H, s, C29-*H*), 8.00 (1H, s, C32-*H*), 7.51 (1H, d, *J* 9.0 Hz, N19-*H*), 5.33 (1H, d, *J* 10 Hz, N4-*H*), 4.87 (1H, dd, *J* 9.0, 7.0 Hz, C5-*H*), 4.75 (1H, dd, *J* 9.0, 5.5 Hz, C20-*H*), 3.78 (3H, s, OCH_3), 2.91 (3H, s, C30-CH_3), 2.75 (3H, s, C33-CH_3), 2.26 (1H, s, C35-*H*), 2.02 (1H, br. s., C26-*H*), 1.61-.1.56 (1H, m, C37-*H*)1.46 (9H, s, C(CH_3)$_3$), 1.34 - 1.25 (1H, m, C37-*H*), 1.03 - 0.95 (9H, m, C26(CH_3)$_2$, C36-H_3, C38-H_3)**;** δ_C (125 MHz, $CDCl_3$) 172.3 (*C21*), 165.6 (*C6*), 162.3 (*C12*), 161.6 (*C18*), 154.3 (*C15*), 153.6 (*C33*), 153.3 (*C9*), 148.2 (*C30*), 143.8 (*C14*), 139.0 (*C29*-H), 130.8 (*C11*), 130.0, 129.9 (*C8*, *C17*),

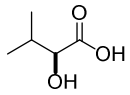
120.1 (*C32*-H), 80.1 (*C*(CH_3)$_3$), 56.2 (*C20*), 54.4 (*C5*), 52.1 (O*C*H_3), 38.0 (*C35*), 33.1 (*C26*), 28.3 (C(*C*H_3)$_3$), 25.3 (*C*37), 18.8, 18.1 (C26(*C*H_3)$_2$), 15.6 (*C*36), 12.2, 11.9, 11.5 (C30-*C*H_3, C33-*C*H_3, *C*38). Missing one quaternary carbon; *m/z* (ESI+) 679.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{31}H_{40}N_6O_8SNa$: 679.2521, found 679.2522 $[M+Na]^+$.

Hexapeptide hydrochloride 4.54



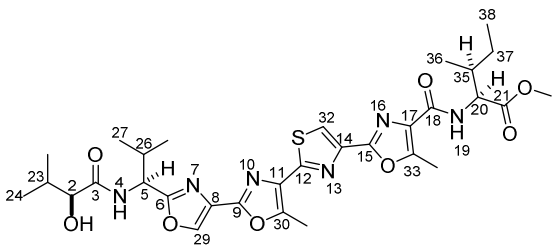
A solution of the Boc-protected hexapeptide **4.53** (89 mg, 0.14 mmol) in methanolic hydrochloride (2M, 2.1 mL) was stirred at room temperature for 2 hours. The mixture was concentrated *in vacuo* to give the deprotected amine hydrochloride salt **4.54** as an amorphous cream solid (80 mg, 0.14 mmol, 99 %); $[\alpha]_D^{28}$ +3.50 (c = 0.495, MeOH); υ_{max}/cm^{-1} (neat) 2966 (NH), 1738 (ester C=O), 1663, 1627 (amide C=O), 1512 (NH); δ_H (700 MHz, MeOD) 8.73 (1H, s, C29-*H*), 8.27 (1H, s, C32-*H*), 4.56 - 4.61 (2H, m, C5-*H*, C20-*H*), 3.80 - 3.76 (3H, m, OC*H*$_3$), 2.89 - 2.86 (3H, m, C30-C*H*$_3$), 2.67 (3H, s, C33-C*H*$_3$), 2.45 (1H, oct. *J* 6.5 Hz, C26-*H*), 2.07 – 2.00 (1H, m, C35-*H*), 1.58 (1H, dqd, *J* 14.5, 7.5, 4.8 Hz, C37-*H*), 1.37 - 1.30 (1H, m, C37-*H*), 1.16 (3H, d, *J* 6.5 Hz, C26-C*H*$_3$), 1.05 (3H, d, *J* 6.5 Hz, C26-C*H*$_3$), 1.00 (3H, d, *J* 6.5 Hz, C36-*H*$_3$), 0.98 (3H, t, *J* 7.5 Hz, C38-*H*$_3$); δ_C (175 MHz, MeOD) 172.0 (*C21*), 162.0, 161.9 (*C6*, *C12*), 160.7 (*C18*), 154.7, 154.1, 153.1 (*C15*, *C33*, *C9*), 148.6 (*C30*), 143.8 (*C14*), 140.8 (*C29*-H), 130.4, 130.0, 129.9 (*C8*, *C17*, *C11*), 120.9 (*C32*-H), 56.4 (*C20*), 53.9 (*C5*), 51.2 (O*C*H_3), 37.3 (*C35*), 33.1 (*C26*), 25.3 (*C*37), 17.4, 16.7 (C26(*C*H_3)$_2$), 14.6 (*C*36), 10.5, 10.4, 10.3 (C30-*C*H_3, C33-*C*H_3, *C*38); *m/z* (ESI+) 579.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{26}H_{33}N_6O_6S$: 557.2177, found 557.2189 $[M-Cl]^+$.

(*S*)-2-Hydroxy-3-methylbutanoic acid 4.55



Prepared according to the method of Bauer *et al*.[36] A solution of L-valine (1.0 g, 8.54 mmol) in dilute aqueous sulphuric acid (0.5M, 34 mL, 17.0 mmol) was cooled to 0 °C. A solution of sodium nitrate (3.53 g, 51.2 mmol) in water (11.5 mL) was added dropwise, and the reaction mixture was stirred at 0-5 °C for a further 30 minutes and at room temperature overnight. The reaction mixture was extracted with Et_2O (5 x 10 mL). The combined organic extracts were washed with saturated aqueous NaCl solution (20 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give yellow crystals. These were recrystallised from pet. ether to give hydroxyacid **4.55** as bright white crystals (0.31 g, 2.62 mmol, 31 %); m.p. 65 - 66 °C (lit.[37] 65 - 66 °C); $[\alpha]_D^{26}$ +14.0 (c = 1.01, $CHCl_3$) (lit.[36] $[\alpha]_D^{20}$ +17.3 (c = 1.06, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3414 (OH), 1703 (acid C=O); δ_H (400 MHz, $CDCl_3$) 6.05 (1H, br.s, CO_2H), 4.16 (1H, d, *J* 3.5 Hz, C*H*OH), 2.17 (1H, septd, *J* 6.5, 3.5 Hz, C*H*$(CH_3)_2$), 1.07 (3H, d, *J* 6.5 Hz, CH(C*H*$_3)_2$), 0.93 (3H, d, *J* 7.0 Hz, CH(C*H*$_3)_2$); δ_C (100 MHz, $CDCl_3$) 179.4 (*C*O_2H), 74.8 (C*H*OH), 32.0 (*C*H$(CH_3)_2$), 18.8 (CH(*C*H$_3)_2$), 15.9 (CH(*C*H$_3)_2$); *m/z* (ESI-) 117.1 ([M-H] 100%); HR-ESIMS: calculated for $C_5H_9O_3$: 117.0557, found 117.0554 $[M+H]^+$. The data are consistent with that previously reported.[38]

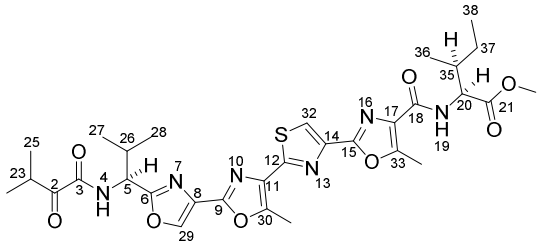
Heptapeptide 4.56



A solution of the (2*S*)-2-hydroxy-3-methylbutanoic acid, **4.55**, (0.08 g, 0.68 mmol) and HOBt (88 %, 0.021 g, 0.14 mmol) in EtOH (3 mL) was stirred at room temperature for 15 minutes before being added to a solution of the amine hydrochloride salt **4.54** (80 mg, 0.14 mmol) in EtOH (2 mL). The reaction mixture

was cooled to 0-5 °C and *N*-methylmorpholine (0.12 mL, 1.1 mmol) was added. After 15 minutes at 0-5 °C, EDCI (0.16 g, 0.68 mmol) was added and the reaction mixture allowed to reach room temperature overnight. The mixture was acidified with pH 2 buffer and EtOH removed *in vacuo*. The mixture was extracted with EtOAc (4 x 5 mL), and the combined organic extracts were washed with water (5 mL), saturated aqueous $NaHCO_3$ solution (5 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the crude heptapeptide. The alcohol **4.56** was obtained by silica chromatography (98:2:0.2 EtOAc:MeOH:AcOH) as an amorphous solid (72 mg, 0.11 mmol, 81 %); $[\alpha]_D^{26}$ -50.2 (c = 0.53, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3432 (NH), 3265 (OH), 1740 (ester C=O), 1648 (amide C=O), 1507 (NH); δ_H (500 MHz, $CDCl_3$) 8.20 (1H, s, C29-*H*), 7.97 (1H, s, C32-*H*), 7.61 (1H, d, *J* 9.5 Hz, N4-*H*), 7.49 (1H, d, *J* 9.0 Hz, N19-*H*), 5.22 (1H, dd, *J* 9.5, 7.0 Hz, C5-*H*), 4.73 (1H, dd, *J* 9.0, 5.5 Hz, C20-*H*), 4.29 – 4.26 (1H, m, C2-*H*), 3.77 (3H, s, OC*H*$_3$), 2.88 (3H, s, C30-C*H*$_3$), 2.74 - 2.71 (3H, m, C30-C*H*$_3$), 2.39 - 2.24 (2H, m, C23-*H*, C26-*H*), 2.05 - 1.96 (1H, m, 1H, m, C35-*H*), 1.62 - 1.51 (1H, m, C37-*H*), 1.34 - 1.23 (1H, m, C37-*H*), 1.06 - 0.90 (18H, m, C24-C*H*$_3$, C25-C*H*$_3$, C27-C*H*$_3$, C28-C*H*$_3$, C36-C*H*$_3$, C38-C*H*$_3$); δ_C (125 MHz, $CDCl_3$) 173.4 (*C3*), 172.2 (*C21*), 165.6 (*C6*), 162.1 (*C12*), 161.6 (*C18*), 165.2 (*C15*), 153.6 (*C22*), 153.0 (*C9*), 148.3 (*C30*), 143.8 (*C14*), 138.5 (*C29*-H), 130.8 (*C11*), 129.9, 129.8 (*C8*, *C17*), 120.2 (*C32*-H), 76.4 (*C2*-H), 56.2 (*C20*), 52.3, 52.1 (*C5*, O*C*H$_3$), 38.0 (*C35*), 32.9, 31.7 (*C23*, *C26*), 25.3 (*C37*), 19.2, 19.0, 18.5, 15.6, 15.5 (*C24*, *C25*, *C27*, *C28*, *C36*) 12.1, 11.9, 11.5 (C30-*C*H$_3$, C33-*C*H$_3$, *C38*); *m/z* (ESI+) 679.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{31}H_{40}N_6O_8SNa$: 679.2521, found 679.2528 $[M+Na]^+$.

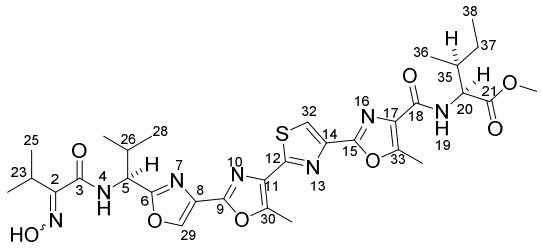
Ketone 4.57



A solution of alcohol **4.56** (57 mg, 0.087 mmol) in dry CH_2Cl_2 (3 mL) and dry DMSO (0.06 mL) was cooled to 0-5°C under a nitrogen atomosphere. $^{i}Pr_2NEt$ (0.12 mL, 0.69 mmol) and SO_3.pyr (83 mg, 0.52 mmol) were added sequentially, and the reaction stirred at 0-5°C for 3 hours. The mixture was acidified with pH 2 buffer and the phases were separated. The aqueous phase was further extracted with CH_2Cl_2 (3 x 3 mL), and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give a yellow gum. This was purified by silica chromatography (50% EtOAc: pet. ether) to give ketone **4.57** as a bright white solid (37 mg, 0.57 mmol, 65%); m.p. 145-146 °C; $[\alpha]_D^{27}$ -30.7 (c = 0.52, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3406 (NH), 1739 (ester C=O), 1670 (ketone C=O), 1628 (amide C=O), 1511 (NH); δ_H (400 MHz, $CDCl_3$) 8.25 (1H, s, C29-*H*), 8.00 (1H, s, C32-*H*), 7.59 (1H, d, *J* 9.5 Hz, N4-*H*), 7.50 (1H, d, *J* 9.0 Hz, N19-*H*), 5.13 (1H, dd, *J* 9.5, 6.5 Hz, C5-*H*), 4.74 (1H, dd, *J* 9.0, 6.0 Hz, C20-*H*), 3.75 - 3.82 (3H, m, OC*H*$_3$), 3.59 (1H, spt, *J* 7.0 Hz, C23-*H*), 2.91 (3H, s, C30-C*H*$_3$), 2.73 - 2.79 (3H, m, C30-C*H*$_3$), 2.31 - 2.45 (1H, m, C26-*H*), 1.93 - 2.09 (1H, m, C35-*H*), 1.51 - 1.63 (1H, m, C37-*H*), 1.25 - 1.36 (1H, m, C37-*H*), 1.18 (3H, d, *J* 6.5 Hz, C25-*H*$_3$), 1.15 (3H, d, *J* 7.0 Hz, C25-*H*$_3$), 0.94 - 1.03 (12H, m, C27-*H*$_3$, C28-*H*$_3$, C36-*H*$_3$, C38-*H*$_3$); δ_C (125 MHz, $CDCl_3$) 201.5 (*C2*), 172.3 (*C21*), 164.0 (*C6*), 162.3 (*C12*), 161.6 (*C3*), 159.5 (*C18*), 154.3 (*C15*), 153.6 (*C33*), 153.1 (*C9*), 148.3 (*C30*), 143.8 (*C14*), 138.7 (*C29*-H), 130.9 (*C11*), 130.2 (*C8*), 130.0 (*C17*), 120.2 (*C32-H*), 56.1 (*C20*-H), 53.0 (*C5*-H), 52.1 (CO_2CH_3), 38.0 (*C35*-H), 34.3 (*C23*-H), 32.9 (*C26*-H), 25.3 (*C37*-H_2), 18.9, 18.2, 17.8, 17.7 (*C24*, *C25*, *C27*, *C28*, *C36*), 12.2, 11.9, 11.5 (C30-*C*H_3, C33-*C*H_3, *C38*);

m/z (ESI+) 677.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{31}H_{38}N_6O_8SNa$: 677.2364, found 677.2352 $[M+Na]^+$.

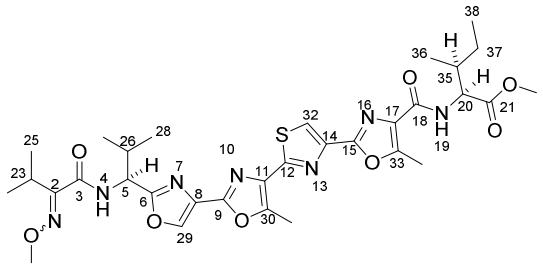
Azolemycin A 4.01



Hydroxylamine hydrochloride (3.0 mg, 0.04 mmol) and pyridine (3.3 μL, 0.04 mmol) were added to a stirred solution of ketone **4.57** (9 mg, 0.014 mmol) in MeOH (0.6 mL) and $CHCl_3$ (0.6 mL) under a nitrogen atmosphere. After 6 hours at room temperature, hydroxylamine hydrochloride (3.0 mg, 0.04 mmol) and pyridine (3.3 μL, 0.04 mmol) were added and the reaction was stirred at room temperature overnight. The reaction mixture was reduced *in vacuo* and the residue partitioned between pH 4 buffer (5 mL) and CH_2Cl_2, and the phases were separated. The aqueous phase was further extracted with CH_2Cl_2 (3 x 2 mL) and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give the crude oxime. This was purified by silica chromatography (50% EtOAc: pet ether) to give azolemycin A, **4.01**, as a mix of *E/Z* stereoisomers (8 mg, 0.012 mmol, 85 %); $[\alpha]_D^{25}$ +6.1 (c = 0.17, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3402 (NH), 3316-3274 (broad, O-H), 1741 (ester C=O), 1668 (C=N), 1629 (amide C=O), 1513 (NH); δ_H (700 MHz, $CDCl_3$) 9.37 (1H, br. s., NO*H*), 8.26 (1H, m, C29-*H*), 8.00 (1H, m, C32-*H*), 7.60 (1H, d, *J* 10.0 Hz, N4-*H*), 7.51 (1H, d, *J* 9.0 Hz, N19-*H*), 5.25 (1H, dd, *J* 10.0, 6.0 Hz, C5-*H*), 4.75 (1H, dd, *J* 9.0, 5.5 Hz, C20-*H*), 3.78 (3H, s, OCH_3), 3.50 (1H, sept, *J* 7.0 Hz, C23-*H*), 2.90 (3H, s, C30-CH_3), 2.75 (3H, s, C33-CH_3), 2.36 - 2.30 (1H, m, C26-*H*), 2.05 – 1.99 (1H, m, C35-*H*), 1.57 (1H, dqd, *J* 15.0, 7.5 Hz, 5.0 Hz, C37-H_2), 1.31 (1H, d, *J* 7.5 Hz, C25-CH_3), 1.30 – 1.23 (4H, m, C25-H_3, C37-H_2), 1.02 (3H, d, *J* 7.0 Hz, C28-CH_3), 1.00 (3H, d, *J* 7.0 Hz, C28-CH_3), 0.98 (3H, d, *J* 7.0 Hz, C36-CH_3),

0.97 (3H, t, *J* 7.5 Hz, C38-CH_3); Minor stereoisomer peaks at 5.34 (1H, dd, *J* 9.0, 6.0 Hz, C5-*H*), 2.97 - 3.02 (1H, m, C23-*H*), 2.43 - 2.37 (1H, m, C26-*H*), 1.19 - 1.17 (3H, m, C25-CH_3), 1.15 - 1.17 (1H, m, C25-CH_3); δ_C (175 MHz, $CDCl_3$) 172.3 (*C21*), 165.6 (*C6*), 163.8 (*C3*), 162.3 (*C12*), 161.6 (*C18*), 158.8 (*C2*), 154.3 (*C15*), 153.7 (*C33*), 152.9 (*C9*), 148.3 (*C30*), 143.8 (*C14*), 138.6 (*C29*-H), 130.9 (*C11*), 129.9, 129.8 (*C17*, *C8*), 120.2 (*C32-H*), 56.2 (*C20*-H), 52.6 (*C5*-H), 52.1 (CO_2CH_3), 38.0 (*C35*-H), 32.9 (*C26*-H), 25.8 (*C23*-H), 25.3 (*C37*-H_2), 18.9, 18.7, 18.5, 18.3, 15.6 (*C24*, *C25*, *C27*, *C28, C36*), 12.1, 11.8, 11.5 (C30-*C*H_3, C33-*C*H_3, *C38*); *m/z* (ESI-) 668.2 ([M-H] 100%); HR-ESIMS: calculated for $C_{31}H_{39}N_7O_8SNa$: 692.2473, found 692.2479 $[M+Na]^+$, calculated for $C_{31}H_{38}N_7O_8S$: 668.2508, found 668.2470 $[M-H]^-$. The data are consistent with the natural product.

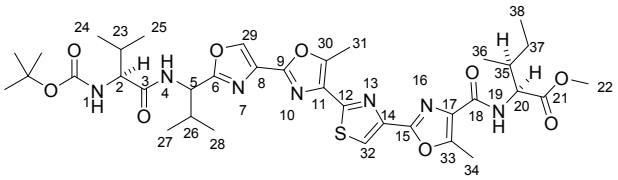
Azolemycin B 4.02



Methoxyamine hydrochloride (3.8 mg, 0.046 mmol) and pyridine (3.7 μL, 0.046 mmol) were added to a stirred solution of ketone **4.57** (10 mg, 0.015 mmol) in MeOH (0.5 mL) and $CHCl_3$ (0.2 mL) under a nitrogen atmosphere. After 4 hours, the reaction mixture was reduced *in vacuo* and the residue partitioned between pH 4 buffer (3 mL) and CH_2Cl_2 (2 mL). The phases were separated and the aqueous phase further extracted with CH_2Cl_2 (3 x 2 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. Azolemycin B, **4.02**, was obtained as a mix of *E/Z* stereoisomers by silica chromatography (50% EtOAc: pet. ether) as a white solid (9 mg, 0.013 mmol, 87 %); $[\alpha]_D^{21}$ +27.6 (c = 0.12, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3414 (NH), 1741 (ester C=O), 1672 (C=N), 1630 (amide C=O), 1509 (NH); δ_H (400 MHz, $CDCl_3$) 8.24 (1H, s, C29-*H*), 8.00 (1H, s, C32-*H*), 7.51 (1H, d, *J* 9.0 Hz, N19-

H), 7.36 (1H, d, *J* 8.5 Hz, N4-*H*), 5.29 (1H, dd, *J* 8.5, 6.5 Hz, C5-*H*), 4.75 (1H, dd, *J* 9.0, 5.5 Hz, C20-*H*), 3.95 (3H, s, NOCH_3), 3.78 (3H, s, CO_2CH_3), 2.98 (1H, sept, *J* 7.0 Hz, C23-*H*), 2.91 (3H, s, C30-CH_3), 2.75 (3H, s, C33-CH_3), 2.42 - 2.32 (1H, m, C26-*H*), 2.00 – 1.89 (1H, m, C35-*H*), 1.52 – 1.45 (1H, m, C37-H_2), 1.36 - 1.25 (3H, m, C37-H_2), 1.16 (3H, d, *J* 7.0 Hz, C25-H_3), 1.14 (3H, d, *J* 7.0 Hz, C25-H_3), 1.05 - 0.94 (12H, m, C36-CH_3,C38-CH_3, C28-CH_3, C28-CH_3); Minor stereoisomer peaks at 5.18 (1H, dd, *J* 9.5, 7.0 Hz, C5-*H*), 4.00 (3H, s, NOCH_3), 3.45 - 3.35 (1H, m, C23-*H*), 1.23 (3H, d, *J* 7.0 Hz, C25-CH_3), 1.22 (3H, d, *J* 7.0 Hz, C25-CH_3); During the time taken to resubmit the compound for further NMR experiments, the sample isomerized to give predominately the other stereoisomer. δ_H (700 MHz, $CDCl_3$) 8.24 (1H, s, C29-*H*), 8.00 (1H, s, C32-*H*), 7.51 (1H, d, *J* 9.0 Hz, N19-*H*), 7.27 – 7.27 (1H, m, N4-*H*), 5.18 (1H, dd, *J* 9.0, 7.0 Hz, C5-*H*), 4.75 (1H, dd, *J* 9.0, 5.5 Hz, C20-*H*), 4.00 (3H, s, NOCH_3), 3.78 (1H, s, CO_2CH_3), 3.40 (1H, sept, *J* 7.0 Hz, C23-*H*), 2.91 (3H, s, C30-CH_3), 2.75 (3H, s, C33-CH_3), 2.35 (1H, oct, *J* 7.0 Hz, C26-*H*), 2.05 - 1.99 (1H, m, C35-*H*), 1.60 - 1.53 (1H, m, C37-H_2), 1.33 - 1.26 (1H, m, C37-H_2), 1.23 (3H, d, *J* 7.0 Hz, C25-H_3), 1.22 (3H, d, *J* 7.0 Hz, C25-H_3), 1.03 (3H, d, *J* 7.0 Hz, C28-CH_3), 1.00 (3H, d, *J* 6.5 Hz, C28-CH_3), 0.97 (3H, t, *J* 7.5 Hz, C38-H_3), 0.96 (3H, d, *J* 6.5 Hz, C35-H_3); δ_C (175 MHz, $CDCl_3$) 172.3 (*C21*), 165.1 (*C6*), 162.7 (*C3*), 162.3 (*C12*), 161.6 (*C18*), 157.1 (*C2*), 154.3 (*C15*), 153.6 (*C33*), 153.3 (*C9*), 148.3 (*C30*), 143.8 (*C14*), 138.6 (*C29*-H), 130.8 (*C11*), 130.0, 129.9 (*C17*, *C8*), 120.1 (*C32-H*), 63.0 (NO*C*H_3), 56.1 (*C20*-H), 52.6 (*C5*-H), 52.1 (CO_2CH_3), 38.0 (*C35*-H), 33.0 (*C26*-H), 25.9 (*C23*-H), 25.3 (*C37*-H_2), 19.0, 18.6, 18.5, 15.6 (*C24*, *C25*, *C27*, *C28, C36*), 12.2, 11.9, 11.5 (C30-*C*H_3, C33-*C*H_3, *C38*); *m/z* (ESI+) 706.2 ([M+H] 100%); HR-ESIMS: calculated for $C_{32}H_{41}N_7O_8SNa$: 706.2630, found 706.2625 $[M+Na]^+$. The data are consistent with that obtained for the natural product.

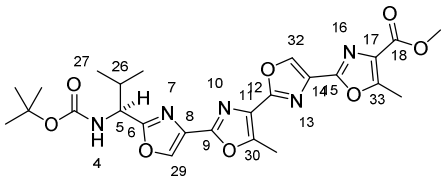
Heptapeptide 4.58



A solution of Boc-Val-OH (79 mg, 0.37 mmol) and HOBt (88 %, 11 mg, 0.08 mmol) in EtOH (2 mL) was stirred for 5 minutes and added to a solution of amine hydrochloride **4.54** (44 mg, 0.075 mmol) in EtOH (1 mL). The solution was cooled to 0 ˚C and *N*-methyl morpholine (0.07 mL, 0.60 mmol) added. After 15 minutes, EDCI (86 mg, 0.45 mmol) was added and the reaction mixture was stirred to room temperature overnight. pH 2 buffer (3 mL) was added and the EtOH removed *in vacuo*. The mixture was extracted with EtOAc (4 x 5 mL), and the combined organic solvents were dried over Na_2SO_4 and concentrated *in vacuo.* The residue was purified by silica chromatography (EtOAc) to give a white solid. Trituration of this with Et_2O (3 x 5 mL) gave heptapeptide **4.58** as a bright white crystalline solid (34 mg, 0.045 mmol, 60 %); m.p. 201 – 202 ˚C; $[\alpha]_D^{28}$ -24.5 (c = 0.70, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3333, 3257 (NH), 1734 (ester C=O), 1686, 1653 (amide C=O), 1516 (NH)**;** δ_H (700 MHz, $CDCl_3$) 8.23 (1H, s, C29-*H*), 8.00 (1H, s, C32-*H*), 7.51 (1H, d, *J* 9.0 Hz, N19-*H*), 6.69 (1H, d, *J* 8.5 Hz, N4-*H*), 5.19 (1H, dd, *J* 9.0, 6.5 Hz, C5-*H*), 5.03 (1H, d, *J* 8.0 Hz, N1-*H*), 4.74 (1H, dd, *J* 9.0, 5.5 Hz, C20-*H*) 3.93 (1H, dd, *J* 8.5, 6.5 Hz, C2-*H*), 3.77 (3H, s, OC*H*$_3$), 2.91 (3H, s, C30-C*H*$_3$), 2.75 (3H, s, C33-C*H*$_3$), 2.32 (1H, oct, *J* 6.5 Hz, C26-*H*), 2.21 - 2.13 (1H, m, C23-*H*), 2.09 - 1.94 (1H, m, C35-*H*), 1.60 - 1.54 (1H, m, C37-*H*$_2$), 1.45 (9H, s, C(C*H*$_3$)$_3$), 1.33 - 1.26 (1H, m, C37-*H*$_2$), 1.05 - 0.91 (18H, m, 2 x C25-*H*$_3$, 2 x C27-*H*$_3$, C36-*H*$_3$, C38-*H*$_3$); δ_C (175 MHz, $CDCl_3$) 172.3 (*C21*), 171.6 (*C3*), 164.8 (*C6*), 162.3 (*C12*), 161.6 (*C18*), 155.9 (*C*$O_2{}^t$Bu), 154.3 (*C15*), 153.6 (*C33*), 153.2 (*C9*), 148.2 (*C30*), 143.8 (*C14*), 138.6 (*C29*-H), 130.8 (*C11*), 130.1, 129.5 (*C8*, *C17*), 120.1 (*C32*-H), 80.1 (*C*$(CH_3)_3$), 60.3 (*C2*), 56.1 (*C20*), 52.8 (*C5*), 52.1 (O*C*H_3), 38.1 (*C35*), 32.8 (*C26*), 30.4 (*C23*), 28.3 (*C*$(CH_3)_3$), 19.4, 18.8, 18.2, 17.9, 15.6 (2 x *C24*-H_3, 2 x *C27*-H_3, *C36*-H_3), 12.2, 11.9,

(C30-*C*H_3, C33-*C*H_3), 11.5 (*C38*-H_3); *m/z* (ESI+) 778.3 ([M+Na] 100%); HR-ESIMS: calculated for $C_{36}H_{49}N_7O_9SNa$: 778.3205, found 778.3191 $[M+Na]^+$.

Tetraoxazole Methyl Ester 4.59

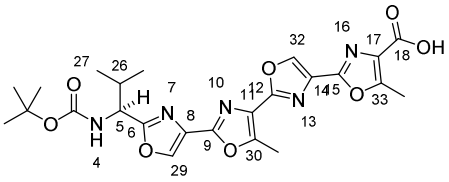


A solution of pentapeptide alcohol **4.30** (0.10 g, 0.19 mmol) in dry CH_2Cl_2 (20 mL) was cooled to -78 ˚C under nitrogen. (Diethylamino)sulfur trifluoride (0.05 mL, 0.35 mmol) was added and the reaction mixture was stirred at -78 ˚C for 2 hours, then at room temperature for 30 minutes. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (10 mL) and was stirred for a further 15 minutes. The phases were separated and the aqueous phase was further extracted with CH_2Cl_2 (2 x 10 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give the crude oxazoline as a bright yellow oil (0.10 g), which was used immediately without further purification.

A solution of the oxazoline (0.10g, 0.19 mmol) in CCl_4 (0.5 mL), MeCN (0.7 mL) and pyridine (0.7 mL) was cooled to 0 ˚C under nitrogen. 1, 8-Diazabicyclo[5.4.0]undec-7-ene (0.12 mL, 0.81 mmol) was added and the reaction mixture was allowed to reach room temperature over 72 hours. The reaction mixture was quenched with pH 2 buffer (5 mL) and extracted with CH_2Cl_2 (3 x 5 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give the crude oxazole. Purification by silica chromatography (5 % MeOH: CH_2Cl_2) gave the oxazole **4.59** as a sticky colourless oil (90 mg, 0.17 mmol, 89 %); $[\alpha]_D^{25}$ -38.3 (c = 0.87, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3330 (NH), 1712 (ester C=O), 1682 (amide C=O), 1523 (NH); δ_H (400 MHz, $CDCl_3$) 8.39 (1H, s, C32-*H*), 8.28 (1H, s, C29-*H*), 5.32 (1H, d, *J* 9.0 Hz, N4-*H*), 4.84 (1H, dd, *J* 9.0, 6.0 Hz, C5-*H*), 3.94 (3H, s, OCH_3), 2.83 (3H, s, C30-CH_3), 2.72 (3H, s, C30-CH_3), 2.29 - 2.18 (1H, m, C36-*H*), 1.44 (9H, s, C(CH_3)$_3$), 1.02 - 0.91 (6H, m, 2 x C27-H_3); δ_C (100 MHz, $CDCl_3$) 165.5 (*C6*),

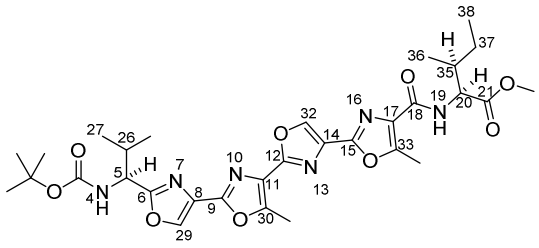
162.5 (*C18*), 156.7, 156.6 (*C12*, *C33*), 166.4, 154.0, 152.9, 151.3 (*C*O_2^{t}Bu, *C9, C15*, *C30*), 138.8, 138.6 (*C29*-H, *C32*-H), 131.0 (*C8*), 129.8 (*C14*), 128.4 (*C17*), 125.7 (*C11*), 80.1 (*C*$(CH_3)_3$), 54.3 (*C5*-H), 52.1 (O*C*H_3), 33.0 (*C26*-H), 28.3 (C(*C*H_3)$_3$), 18.7, 18.0 (2 x *C27*-H_3), 12.1, 11.9 (C30-*C*H_3, C33-*C*H_3); *m/z* (ESI+) 550.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{25}H_{29}N_5O_8Na$: 550.1908, found 550.1909 $[M+Na]^+$.

Tetraoxazole Carboxylic Acid 4.60



A solution of LiOH (0.04 g, 1.71 mmol) in water (1.3 mL) was added to a solution of tetraoxazole methyl ester **4.59** (90 mg, 0.17 mmol) in THF (7.5 mL), CH_2Cl_2, (1.0 mL) and MeOH (7.5 mL). After 6 hours at room temperature, the reaction mixture was acidified with pH 2 buffer and the organic solvents removed *in vacuo*. EtOAc (10 mL) was added and the phases were separated. The aqueous phase was further extracted with EtOAc (3 x 5 mL) and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give acid **4.60** as a sticky colourless oil (60 mg, 0.11 mmol, 67 %); $[\alpha]_D^{23}$ -15.9 (c = 0.15, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3338 (NH), 3317 (O-H), 1701 (ester C=O), 1655 (amide C=O), 1524 (NH); δ_H (400 MHz, $CDCl_3$) 8.40 (1H, s, C32-*H*), 8.30 (1H, s, C29-*H*), 5.39 (1H, d, *J* 9.5 Hz, N4-*H*), 4.86 (1H, dd, *J* 9.0, 6.0 Hz, C5-*H*), 2.84 (3H, s, C30-C*H*$_3$), 2.76 (3H, s, C33-C*H*$_3$), 2.29 - 2.19 (1H, m, C26-*H*), 1.49 - 1.44 (9H, m, C(C*H*$_3$)$_3$), 0.99 - 0.93 ppm (6H, m, 2 x C27-C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 164.6 (*C6*), 164.1 (*C*O_2H), 156.5 (*C33*), 155.8 (*C12*), 154.4, 152.9, 152.0, 150.4 (*C9, C11*, *C15*, *C30*), 137.8, 137.8 (*C29*-H, *C32*-H), 129.8 (*C14*), 128.7 (*C8*), 127.1 (*C17*), 124.6 (*C11*), 79.1 (*C*$(CH_3)_3$), 53.3 (*C5*-H), 32.0 (*C26*-H), 27.3 (C(*C*H_3)$_3$), 17.7, 17.0 (2 x C27-*C*H_3), 11.2, 10.9 (C30-*C*H_3, C33-*C*H_3); *m/z* (ESI+) 536.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{24}H_{27}N_5O_8Na$: 536.1752, found 536.1751 $[M+Na]^+$.

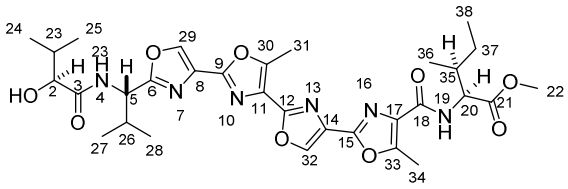
Tetraoxazole Hexapeptide 4.61



A solution of tetraoxazole carboxylic acid **4.60** (59 mg, 0.11 mmol) and HOBt (88 %, 4 mg, 0.02 mmol) in EtOH (3 mL) was stirred at room temperature for 15 minutes. L-Isoleucine methyl ester hydrochloride (52 mg, 0.29 mmol) was added and the solution was cooled to 0 ˚C. *N*-Methyl morpholine (0.08 mL, 0.75 mmol) was added followed, after 15 minutes, by EDCI (33 mg, 0.17 mmol), and the mixture was allowed to reach room temperature overnight. pH 2 buffer (5 mL) was added and mixture was extracted with EtOAc (5 x 5 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ solution (5 mL), water (5 mL) and brine (5 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by silica chromatography (2% MeOH: CH_2Cl_2) gave the hexapeptide as a clear pale yellow oil (38 mg, 0.06 mmol, 54 %); $[\alpha]_D^{28}$ -18.2 (c = 0.56, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3346 (NH), 3317 (O-H), 1739 (ester C=O), 1667 (amide C=O), 1511 (NH); δ_H (600 MHz, $CDCl_3$) 8.30, 8.29 (1H, s, C29-*H*; 1H, s, C32-*H*), 7.47 (1H, d, *J* 9.0 Hz, N19-*H*), 5.32 (1H, d, *J* 9.0 Hz, N4-*H*), 4.85 (1H, dd, *J* 9.0, 6.0 Hz, C5-*H*), 4.73 (1H, dd, *J* 9.0, 5.3 Hz, C20-*H*), 3.77 (3H, s, OCH_3), 2.86 (3H, s, C30-CH_3), 2.72 (3H, s, C33-CH_3), 2.28 - 2.21 (1H, m, C26-*H*), 2.04 - 1.96 (1H, m, C35-*H*), 1.55 (1H, dqd, *J* 13.0, 8.0, 4.5 Hz, C37-H_2), 1.45 (9H, s, C(CH_3)$_3$), 1.33 - 1.25 (1H, m, C37-H_2), 1.02 - 0.93 (12H, m, 2 x C27-H_3, C36-H_3, C38-H_3); δ_C (150 MHz, $CDCl_3$) 172.2 (*C21*), 165.5 (*C6*), 161.4 (*C18*), 156.9 (*C12*), 155.4 (*C*O_2^{t}Bu), 154.0, 153.7 (*C9*, *C30*), 151.9, 151.4 (*C15*, *C33*), 138.8, 138.3 (*C29*-H, *C32*-H), 131.1, 129.9, 129.7 (*C8*, *C14*, *C17*), 125.7 (*C11*), 80.1 (*C*(CH_3)$_3$), 56.1 (*C20*-H), 54.3 (*C5*-H), 52.1 (O*C*H_3), 38.0 (*C35*-H), 33.0 (*C26*-H), 28.3 (C(*C*H_3)$_3$), 25.3 (*C37*-H_2), 18.7, 18.0 (2 x *C27*-H_3),

15.6 (*C36*-H_3), 12.0, 11.8 (C30-*C*H_3, C33-*C*H_3), 11.5 (*C38*-H_3); *m/z* (ESI+) 663.2 ([M+Na] 100%); HR-ESIMS: calculated for $C_{31}H_{44}N_7O_9$: 658.3195, found 658.3196 $[M+NH_4]^+$.

Tetraoxazole Alcohol 4.63

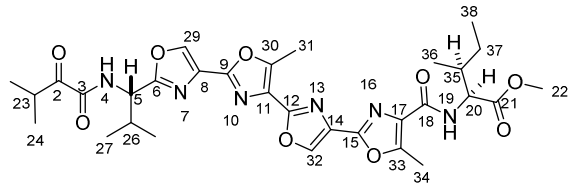


Tetraoxazole **4.61** (38 mg, 0.06 mmol) was dissolved in a solution of acetyl chloride (0.12 mL, 1.8 mmol) and MeOH (0.8 mL), and the reaction mixture stirred at room temperature for 2 hours. The reaction mixture was concentrated *in vacuo* to give the deprotected amine as the hydrochloride salt, **4.62**. *m/z* (ESI+) 563.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{26}H_{32}N_6O_7Na$: 563.2225, found 563.2237 $[M+Na]^+$.

A solution of the alcohol **4.55** (14 mg, 0.12 mmol) and HOBt (88 %, 3 mg, 0.02 mmol) in EtOH (3 mL) was stirred at room temperature for 10 minutes and added to the deprotected amine, **4.62** (0.06 mmol). The mixture was cooled to 0 ˚C, and *N*-methyl morpholine (0.04 mL, 0.38 mmol) was added. After 15 minutes, EDCI (30 mg, 0.14 mmol) was added and the reaction mixture was stirred for 18 hours. pH 2 buffer (3 mL) and EtOAc (3 mL) were added and the phases were separated. The aquoues phase was further extracted with EtOAc (3 x 3 mL), and the combined organic extracts were washed with brine (3 mL) and dried over Na_2SO_4. The alcohol **4.63** was obtained by silica chromatography (2 % MeOH: EtOAc) as a clear oil (24 mg, 0.037 mmol, 62 %); $[\alpha]_D^{28}$ -39.4 (c = 0.34, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3408 (NH), 2966 (O-H), 1740 (ester C=O), 1665 (amide C=O), 1515 (NH); δ_H (700 MHz, $CDCl_3$) 8.30 (1H, s, C32-*H*), 8.27 (1H, s, C29-*H*), 7.48 (1H, d, *J* 9.0 Hz, N19-*H*), 7.37 (1H, d, *J* 9.0 Hz, N4-*H*), 5.21 (1H, dd, *J* 9.0, 6.0 Hz, C5-*H*), 4.73 (1H, dd, *J* 9.0, 5.5 Hz, C20-*H*), 4.10 - 4.15 (1H, m, C2-*H*), 3.77 (3H, s, OCH_3), 3.30 (1H, br. s., O*H*), 2.85 (3H, s, C30-CH_3), 2.72 (3H, s, C33-CH_3), 2.34 (1H, oct, *J* 7.0 Hz, C26-

H), 2.28 - 2.21 (1H, m, C23-*H*), 2.03 – 1.97 (1H, m, C35-*H*), 1.59 - 1.53 (1H, m, C37-H_2), 1.33 - 1.27 (1H, m, C37-H_2), 1.07 – 0.90 (18H, m, 2 x C24-H_3, 2 x C27-H_3, C36-H_3, C38-H_3); δ_C (150 MHz, $CDCl_3$) 173.5 (*C3*), 172.2 (*C21*), 165.3 (*C6*), 161.4 (*C18*), 156.8 (*C12*), 153.9, 153.7 (*C15*, *C33*), 151.8, 151.4 (*C9*, *C30*), 138.9 (*C29*-H), 138.4 (*C32*-H), 131.1 (*C14*), 130.0, 129.7 (*C8*, *C17*), 125.6 (*C11*), 76.5 (*C2*-H), 56.2 (*C20*-H), 52.3, 52.1 (*C5*-H, OCH_3), 37.9 (*C35*-H), 32.6 (*C26*-H), 31.7 (*C23*-H), 25.3 (*C37*-H_2), 19.2, 18.7, 18.2, 15.9, 15.6 (2 x *C27*-H_3, 2 x *C24*-H_3, *C36*-H_3), 12.0, 11.8 (C30-*C*H_3, C33-*C*H_3), 11.5 (*C38*-H_3); *m/z* (ESI+) 663.2 ([M+Na] 100%); HR-ESIMS: calculated for $C_{31}H_{40}N_6O_9Na$: 663.2749, found 663.2754 $[M+Na]^+$.

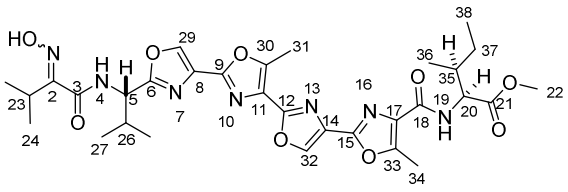
Tetraoxazole Ketone 4.64



A solution of alcohol **4.63** (24 mg, 0.037 mmol) in dry CH_2Cl_2 (1.5 mL) and dry DMSO (0.03 mL, 0.37 mmol) was stirred at 0 ˚C under N_2. Diisopropylethylamine (0.05 mL, 0.30 mmol) was added, followed by SO_3. pyridine complex (35 mg, 0.22 mmol) and the reaction mixture was held at 0 ˚C for 6 hours. The reaction mixture was quenched with pH 2 buffer and the phases were separated. The aqueous phase was further extracted with CH_2Cl_2 (5 x 3 mL) and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. Ketone **4.64** was obtained by silica chromatography as a clear, colourless oil (17 mg, 0.027 mmol, 72 %); $[\alpha]_D^{25}$ -28.0 (c = 0.87, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3401 (NH), 1740 (ester C=O), 1675 (ketone C=O), 1662 (amide C=O), 1513 (NH); δ_H (400 MHz, $CDCl_3$) 8.30 (1H, s, C29-*H*), 8.29 (1H, s, (1H, s, C32-*H*), 7.57 (1H, d, *J* 9.0 Hz, N4-*H*), 7.46 (1H, d, *J* 9.0 Hz, N19-*H*), 5.11 (1H, dd, *J* 9.5, 7.0 Hz, C5-*H*), 4.72 (1H, dd, *J* 9.0, 5.5 Hz, C20-*H*), 3.79 (3H, s, OCH_3), 3.58 (1H, spt, *J* 7.0 Hz, C23-*H*), 2.85 (3H, s, C30-CH_3), 2.74 - 2.70 (3H, m, C33-CH_3), 2.35 (1H, oct, *J* 7.0 Hz, C26-*H*), 2.06 - 1.94 (1H, m, C35-

H), 1.62 - 1.49 (1H, m, C37-H_2), 1.34 - 1.22 (1H, m, C37-H_2), 1.17 (3H, d, *J* 6.5 Hz, C24-H_3), 1.14 (6 H, d, *J* 7.0 Hz, C24-H_3), 1.02 - 0.93 (12H, m, 2 x C27-H_3, C36-H_3, C38-H_3); δ_C (100 MHz, $CDCl_3$) 201.5 (*C2*), 172.2 (*C*O_2Me), 163.9 (*C6*), 161.4 (*C18*), 159.5 (*C3*), 156.9 (*C12*), 153.7, 151.9, 151.5 (*C9*, *C15*, *C30*, *C33*), 139.0 (*C32*-H), 138.3 (*C29*-H), 131.1, 130.1, 129.9 (*C8*, *C14*, *C17*), 125.7 (*C11*), 56.2, (*C20*-H), 53.0 (*C5*-H), 52.1 (O*C*H_3), 38.0 (*C35*-H), 34.3 (*C23*-H), 32.8 (*C26*-H), 25.3 (*C37*-H_2), 18.2, 17.7, 17.6, 15.6 (2 x *C27*-H_3, 2 x *C24*-H_3, *C36*-H_3), 12.0, 11.8 (C30-*C*H_3, C33-*C*H_3), 11.5 (*C38*-H_3); *m/z* (ESI+) 661.1 ([M+Na] 100%); HR-ESIMS: calculated for $C_{31}H_{38}N_6O_9Na$: 661.2592, found 661.2606 $[M+Na]^+$.

Tetraoxazole Analogue of Azolemycin A 4.65



Pyridine (13 µL, 0.017 mmol) and hydroxylamine hydrochloride (12 mg, 0.017 mmol) were added to a solution of the ketone **4.64** (17 mg, 0.027 mmol) in MeOH (0.6 mL) and $CHCl_3$ (0.6 mL). The reaction mixture was stirred under nitrogen for 18 hours, and then concentrated *in vacuo*. The residue was partitioned between CH_2Cl_2 (5 mL) and pH 4 buffer (5 mL). The phases were separated and the aqueous phase was further extracted with CH_2Cl_2 (3 x 2 mL). The combined organic extracts were dried over sodium sulfate and concentrated *in vacuo*. The tetraoxazole analogue of azolemycin A, **4.65**, was obtained by silica chromatography (50 % EtOAc: pet. ether) as a clear colourless oil (6 mg, 0.009 mmol, 33 %); $[\alpha]_D^{28}$ +1.6 (c = 0.19, $CHCl_3$); υ_{max}/cm^{-1} (neat) 3394 (NH), 3304 (OH), 1740 (ester C=O), 1665 (C=N), 1513 (NH); δ_H (700MHz, $CDCl_3$) 8.31 (1H, s, C29-*H*), 8.30 (1H, s, C32-*H*), 7.51 (1H, d, *J* 8.4 Hz, N4-*H*), 7.48 (1H, d, *J* 9.2 Hz, N19-*H*), 5.32 (1H, dd, *J* 8.8, 6.2 Hz, C5-*H*), 4.74 (1H, dd, *J* 9.0, 5.5 Hz, C20-*H*), 3.77 (1H, s, O*C*H_3), 3.01 (1H, sept, *J* 6.9 Hz, C23-*H*), 2.86 (1H, s, C30-CH_3), 2.73 (1H, s, C33-CH_3), 2.38 (1H, oct, *J* 6.2 Hz,

C26-*H*), 2.05 - 1.98 (1H, m, C35-*H*), 1.60 - 1.52 (1H, m, C37-H_2), 1.35 - 1.21 (1H, m, C37-H_2), 1.17 (3H, d, *J* 6.6 Hz, C24-H_3), 1.15 (3H, d, *J* 7.0 Hz, C24-H_3), 1.03 – 0.99 (9H, m, 2 x C27-H_3, C33-H_3), 0.97 (3H, t, *J* 7.3 Hz, C38-H_3); Minor stereoisomer peaks at 5.20 (1H, dd, *J* 9.2, 6.6 Hz, C5-*H*), 3.51 - 3.46 (1H, m, C23-*H*), 2.34 – 2.29 (1H, m, C26-*H*); δ_C (175MHz, $CDCl_3$) 172.2 (*C21*), 164.6 (*C6*), 161.8 (*C18*), 161.5 (*C3*), 157.6 (*C2*), 156.9 (*C9*), 153.8, 153.7 (*C12*, *C33*), 151.9, 151.5 (*C15*, *C30*), 139.0 (*C29*-H), 138.3 (*C32*-H), 131.1 (*C17*), 129.9, 129.8 (*C8*, *C14*), 125.7 (*C11*), 56.1 (*C20*-H), 52.9 (*C5*-H), 52.1 (OCH_3), 38.0 (*C35*-H), 32.8 (*C26*-H), 30.9 (*C23*-H), 25.3 (*C37*-H_2), 20.0, 19.8 (2 x *C24*-H_3), 18.9, 18.4 (2 x *C27*-H_3), 15.6 (C36-H_3), 12.0, 11.8 (C30-CH_3, C33-CH_3), 11.5 (C38-H_3); *m/z* (ESI+) 675.9 ([M+Na] 100%); HR-ESIMS: calculated for $C_{31}H_{39}N_7O_9Na$: 676.2701, found 767.2697 $[M+Na]^+$.

4.11 EXPERIMENTAL REFERENCES

1. P. G. Andersson, D. Guijarro and D. Tanner, *J. Org. Chem.*, 1997, **62**, 7364-7375.
2. H. Aït-Haddou, O. Hoarau, D. Cramailére, F. Pezet, J.-C. Daran and G. G. A. Balavoine, *Chem. - Eur. J.*, 2004, **10**, 699-707.
3. P. M. T. Ferreira, L. S. Monteiro and G. Pereira, *Eur. J. Org. Chem.*, 2008, **2008**, 4676-4683.
4. J. Deeley, A. Bertram and G. Pattenden, *Org. Biomol. Chem.*, 2008, **6**, 1994-2010.
5. A. W. Allan and B. H. Walter, *J. Chem. Soc. C*, 1968, 1397-1399.
6. P. Wipf, A. Cunningham, R. L. Rice and J. S. Lazo, *Bioorg. Med. Chem.*, 1997, **5**, 165-177.
7. K. A. Fennell, U. Mollmann and M. J. Miller, *J. Org. Chem.*, 2008, **73**, 1018-1024.
8. V. du Vigneaud and C. E. Meyer, *J. Biol. Chem.*, 1932, **98**, 295-308.

9. K. Kawasaki, R. Gamble, G. Dupuis, H. Romovacek, H. T. Storey, C. Yanaihara and K. Hofmann, *J. Am. Chem. Soc.*, 1973, **95**, 6815-6824.

10. T. Doi, M. Yoshida, K. Shin-ya and T. Takahashi, *Org. Lett.*, 2006, **8**, 4165-4167.

11. P. Garner and J. M. Park, *J. Org. Chem.*, 1987, **52**, 2361-2364.

12. C. Toniolo, G. M. Bonora, G. R. Sullivan, W. H. Bearden and J. D. Roberts, *J. Org. Chem.*, 1980, **45**, 288-290.

13. A. I. Meyers and F. X. Tavares, *J. Org. Chem.*, 1996, **61**, 8207-8215.

14. M. L. Kantam, M. Annapurna, P. R. Likhar, P. Srinivas, N. Mirzadeh and S. K. Bhargava, *J. Organomet. Chem.*, 2013, **723**, 129-136.

15. C. Paulitz and W. Steglich, *J. Org. Chem.*, 1997, **62**, 8474-8478.

16. E. J. Zylstra, M. W. L. She, W. A. Salamant and J. W. Leahy, *Synlett*, 2007, **2007**, 0623-0627.

17. S. V. Downing, E. Aguilar and A. I. Meyers, *J. Org. Chem.*, 1999, **64**, 826-831.

18. A. Bertram, N. Maulucci, O. M. New, S. M. Mohd Nor and G. Pattenden, *Org. Biomol. Chem.*, 2007, **5**, 1541-1553.

19. P. Loos, C. Ronco, M. Riedrich and H.-D. Arndt, *Eur. J. Org. Chem.*, 2013, **2013**, 3290-3315.

20. H. Otsuka, K. Inouye, M. Kanayama and F. Shinozaki, *Bull. Chem. Soc. Jpn.*, 1965, **38**, 679-680.

21. T. S. Ibrahim, S. R. Tala, S. A. El-Feky, Z. K. Abdel-Samii and A. R. Katritzky, *Synlett*, 2011, **2011**, 2013-2016.

22. M. Trajkovic, Z. Ferjancic and R. N. Saicic, *Tetrahedron: Asymmetry*, 2012, **23**, 602-604.

23. D. J. Chambers, G. R. Evans and A. J. Fairbanks, *Tetrahedron: Asymmetry*, 2005, **16**, 45-55.

24. S. K. Chattopadhyay, S. Biswas and B. K. Pal, *Synthesis*, 2006, **2006**, 1289-1294.

25. M. C. Bagley, K. E. Bashford, C. L. Hesketh and C. J. Moody, *J. Am. Chem. Soc.*, 2000, **122**, 3301-3313.

26. S. B. King and K. B. Sharpless, *Tetrahedron Lett.*, 1994, **35**, 5611-5612.

27. P. Zarantonello, C. P. Leslie, R. Ferritto and W. M. Kazmierski, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 561-565.

28. B. McKeever and G. Pattenden, *Tetrahedron*, 2003, **59**, 2713-2727.

29. G. Videnov, D. Kaiser, C. Kempter and G. Jung, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1503-1506.

30. L. A. Carpino, *J. Org. Chem.*, 1970, **35**, 3971-3972.

31. R. L. E. Furlán, E. G. Mata, O. A. Mascaretti, C. Peña and M. P. Coba, *Tetrahedron*, 1998, **54**, 13023-13034.

32. S. Peña, L. Scarone, E. Manta, L. Stewart, V. Yardley, S. Croft and G. Serra, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 4994-4997.

33. B. Wagner, D. Schumann, U. Linne, U. Koert and M. A. Marahiel, *J. Am. Chem. Soc.*, 2006, **128**, 10513-10520.

34. A. J. Fatiadi, *Synthesis*, 1976, **1976**, 65-104.

35. I. M. Goldman, *J. Org. Chem.*, 1969, **34**, 1979-1981.

36. T. Bauer and J. Gajewiak, *Tetrahedron*, 2004, **60**, 9163-9170.

37. W. R. Li, W. R. Ewing, B. D. Harris and M. M. Joullie, *J. Am. Chem. Soc.*, 1990, **112**, 7659-7672.

38. D. X. Hu, M. O'Brien and S. V. Ley, *Org. Lett.*, 2012, **14**, 4246-4249.

CHAPTER 5: CONCLUSIONS AND FUTURE WORK

5.1 CTLPS AND THIOACIDS

While the synthesis of the ten CTLPs was successfully completed (Chapter 2), neither the linear (Scheme 2), or the block peptide syntheses (Scheme 5) were particularly efficient. As this project continued, we became more aware of the limitations of conventional peptide coupling reagents, with the removal of by-products, solubility issues and side-reactions being a constant theme. In addition we were becoming increasingly concerned with identifying and quantifying epimerization during these reactions. These CTLPs are currently awaiting biological testing, but in the event that any proved to be promising new somatotaxins a more robust synthesis would be required.

While we hoped that thioacids would provide a route for this, the increased levels of epimerization during the formation of the thioester precursors compared to the direct synthesis of the model peptides meant this route was not viable (Chapter 3). In addition, the preparation of the thioesters and subsequent amide bond formation was still reliant on standard peptide coupling reagents. While Albericio *et al*. have described direct block peptide formation from related thioester compounds,[1] the thioesters are still prepared using the methods described by Crich *et al*.[2, 3] Interestingly, Crich does not report any significant epimerization of the C-terminal amino-acids during formation of the thioester, release of the thioacid or subsequent amide-bond formation. Albericio noted ~5% racemisation during the formation of the peptide Boc-Ala-Ala-Gly-Leu-Try-SC_6H_4-NO_2, but reported no or very low epimerization for other thioester peptides. However, as epimerization was identified by the measurement of diastereomers in compounds following purification by preparation reverse-phase HPLC, rather than the crude reaction mixture, it is difficult to say how accurate these are, especially as the diastereomeric ratios are not

measured by comparison with a previously prepared, diastereomerically pure standard.

While it is possible that the isoleucine-containing thioesters prepared in Chapter 3 are not a realistic model for the more natural linear and cyclic peptides synthesized by both Crich and Albericio, the fact that preparing the model peptides using standard PyBOP coupling conditions (Scheme 21) required fewer steps and gave significantly lower levels of epimerization than the equivalent thioester route makes it difficult to consider peptide couplings using thioesters as a viable alternative.

5.2 Enzymes in Peptide Coupling

There is still a need for a general, cheap and epimerisation-free method of peptide bond formation, which we do not believe will be met by this thioester route. The vast array of onium and related coupling agents available to the chemist today suggests that the 'traditional' activating agents are also not the answer,[4] as no one reagent performs well in every situation,[5, 6] and often new peptide coupling agents are limited in scope to the substrates listed in the initial publication.[7]

Enzymes, principally proteases, acylases, amidases, lipases and subtilisin,[6, 8] have been used for peptide synthesis since 1938,[9] and have many apparent advantages over synthetic peptide coupling agents, namely proceeding with no racemization, mild conditions, and high levels of regio- and stereoselectivity.[10] However, reactions involving enzymes have often been limited by substrates, often only tolerating L-amino-acids and their derivatives, and there is a risk of secondary hydrolysis of the growing peptide chain. Promisingly, for the enzyme subtilisin, the substrate limitation has been remedied by the use of organic solvents and extended reaction time, allowing some incorporation of D-amino-acids.[11-13] In addition, a number of acyl-donors have been developed to be specifically recognized by the enzyme, with de Beer *et al*. describing easily accessible *C*-terminal esters that gave high levels of

amide-bond formation without racemization using Alcalase, a commercial form of subtilisin A.[14] Crucially, these did not require the use of coupling agents to synthesise, and were easy to obtain in a chemically and stereochemically pure form. Notably, the guanidophenyl, carboxamidomethyl and trifluroenthyl esters could be themselves prepared using enzymatic catalysis, ensuring no epimerization during the acylation.[15] Where aryl amines were used, simple acids or methyl or benzyl esters could be used for the synthesis of arylamides, providing access to di- and tripeptides.[16]

The use of enzymatic block peptide synthesis is of great interest to us, and it could potentially provide a route to new peptidyl CTLPs with comparative ease.

5.3 ISOLEUCINE

The use of isoleucine-containing molecules as markers to quantify epimerization in both peptide bond formation, and during other reactions, has been of considerable use throughout the total synthesis of azolemycin A (Chapter 4), where a *C*-terminal valine residue was exposed to relatively harsh conditions during the synthesis of the oxazoles and thiazoles. Using isoleucine-containing derivatives as model compounds allowed us to be reasonably confident in retaining the stereointegrity of the valine throughout the synthesis. In addition, the use of isoleucine containing molecules allowed us to accurately predict the relative stereochemistry of the isoleucine residue in azeolymycin A. We hope that the trends shown in Chapter 3 (Tables 2 – 4) will allow for a general method of identifying the relative stereochemistry of isoleucine residues within natural products.

Further ^{13}C NMR studies in different solvents would provide additional support to the assignments made by ^{1}H NMR spectra, especially in compounds where the peaks corresponding to the isoleucine α-C*H* are obscured.

Ideally, we would like to expand this work to encorporate the less common D-isoleucine and L-*allo*-isoleucine, and thus be able to produce model compounds with all four stereoisomers of isoleucine. This would give us the opportunity to validate the stereochemical assignment for more peptide-like molecules, such as the natural product YM-316291 (**Figure 46**). By synthesizing a model peptide, including an L-valine residue and all four stereoisomers of isoleucine it should be possible identify the correct amino-acid stereochemistry by comparison with the ^{1}H NMR of the isolated natural product.

The less common L-*allo*-isoleucine and D-isoleucine are relatively expensive commercially, but we hope to obtain them by use of enzymatic resolution as described by Greenstein *et al.*[17]

5.4 Preparation of Unactivated Oxazoles

The principle obstacle in the synthesis of azolemycin A was the oxidation of the unactivated oxazolines to oxazoles (Scheme 42, Scheme 44). Ultimately, this was achieved using freshly prepared MnO_2 with the best yields observed in the synthesis of **4.09**. Though the yields were low, it is likely they could be improved by optimization of the work-up. This provides a valuable route to isolated, non-conjugated oxazoles. Both azolemycins A and B are currently awaiting biological testing, and the convergent nature of the final iteration of the total synthesis described in Chapter 4 would allow for a range of analogues to be prepared if required.

The comparison between the ^{1}H and ^{13}C NMR spectra of azolemycin A **4.01**, and the tetra-oxazole analogue, **4.65**, highlighted some significant differences between the peaks for protons and carbons associated with, and adjacent to, azole **C** (Figures 56 and 57). It would be useful to compare this data with that of similar mixed azoles (e.g. YM-316291 and IB-01211, **Figure 46**) and also synthesise a number of model

compounds containing a mixture of oxazoles and thiazoles to see if it is possible to pinpoint the relative position of a thiazole within a group of oxazoles. It would have been much more difficult to assign the structure of azolemycin A, and work out the position of the thiazole, if it contained only serine-derived oxazoles, as the methyl groups on the **B** and **D** oxazoles provided useful markers for elucidation of the HMBC NMR, and hence assignment of the quarternary carbons.

Another tool that could be applied to the structural assignment of novel mixed azole natural products is atomic-resolution scanning probe microscopy. This has already been used successfully to validate the structure of a natural product, previously misassigned by NMR spectroscopy.[18] Because of the ability of this technique to image molecules at an atomic level, comparison of atomic force microscopy (AFM) images of azolemycin A, **4.01**, and the tetra-oxazole equivalent, **4.65**, would allow us to confirm it there is any visible difference between a thiazole and an oxazole at this level. This data, when used in conjuction with more traditional techniques such as NMR and mass-spectrometry, could then assist in the initial structural elucidation of new linked polyazole molecules.

5.5 REFERENCES

1. P. Agrigento, F. Albericio, S. Chamoin, I. Dacquignies, H. Koc and M. Eberle, *Org. Lett.*, 2014.
2. D. Crich and I. Sharma, *Angew. Chem., Int. Ed.*, 2009, **48**, 2355-2358.
3. D. Crich and K. Sana, *J. Org. Chem.*, 2009, **74**, 7383-7388.
4. A. El-Faham and F. Albericio, *Chem. Rev. (Washington, DC, U. S.)*, 2011, **111**, 6557-6602.
5. J. Hachmann and M. Lebl, *Pept. Sci.*, 2006, **84**, 340-347.
6. J. R. Dunetz, Y. Xiang, A. Baldwin and J. Ringling, *Org. Lett.*, 2011, **13**, 5048-5051.
7. E. Valeur and M. Bradley, *Chem. Soc. Rev.*, 2009, **38**, 606-631.

8. A. L. Margolin and A. M. Klibanov, *J. Am. Chem. Soc.*, 1987, **109**, 3802-3804.

9. M. Bergmann, H. Fraenkel-Conrat and W. t. c. o. D. G. Doherty, *J. Biol. Chem.*, 1938, **124**, 1-6.

10. C. H. Wong and G. M. Whitesides, *Enzymes in Synthetic Organic Chemistry*, Tarrytown N.Y., 1994.

11. M. V. Sergeeva, V. M. Paradkar and J. S. Dordick, *Enzyme Microb. Technol.*, 1997, **20**, 623-628.

12. A. Ferjancic, A. Puigserver and H. Gaertner, *Appl. Microbiol. Biotechnol.*, 1990, **32**, 651-657.

13. A. L. Margolin, D. F. Tai and A. M. Klibanov, *J. Am. Chem. Soc.*, 1987, **109**, 7885-7887.

14. R. J. A. C. de Beer, T. Nuijens, L. Wiermans, P. J. L. M. Quaedflieg and F. P. J. T. Rutjes, *Org. Biomol. Chem.*, 2012, **10**, 6767-6775.

15. T. Nuijens, C. Cusan, A. C. H. M. Schepers, J. A. W. Kruijtzer, D. T. S. Rijkers, R. M. J. Liskamp and P. J. L. M. Quaedflieg, *J. Mol. Catal. B: Enzym.*, 2011, **71**, 79-84.

16. T. Nuijens, C. Cusan, J. A. W. Kruijtzer, D. T. S. Rijkers, R. M. J. Liskamp and P. J. L. M. Quaedflieg, *J. Org. Chem.*, 2009, **74**, 5145-5150.

17. J. P. Greenstein, L. Levintow, C. G. Baker and J. White, *J. Biol. Chem.*, 1951, **188**, 647-663.

18. L. Gross, F. Mohn, N. Moll, G. Meyer, R. Ebel, W. M. Abdel-Mageed and M. Jaspars, *Nat Chem*, 2010, **2**, 821-825.